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NUTRITION REVIEWS

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Nutrition and Ophthalmology 65
Iron Compounds for Enrichment of Bread 66
A Comparison of Omnivorous and Vegetarian Diets 67
Organic Elements in Mature Dental Enamel 70
Disturbance in Tyrosine Metabolism in Pernicious Anemia 70
A Chick Growth Factor in Cow Manure 71
Cobalt Studies with the Rabbit 73
Dietary Oxalates, Penicillin, and Experimental Tooth Decay 75
Inactivity of Indole-3-Acetic Acid as a "Pellagragenic" Factor in the Rat 77
Osteomalacia Due to Increased Resistance to Vitamin D 79
Fats in Rat Nutrition 80
Nutrition Studies with Germ-Free Animals 83
Riboflavin, Thiamine, and Vitamin A in Dairy Products 86
Vitamin Deficiency and Immunity in Rats 87
Influence of Choline in Megaloblastic Anemias 89
Comparison of Folic Acid and Liver Therapy in Macrocytic Anemias 90
The Site of Conversion of Carotene to Vitamin A 92
Excretion of B-complex Vitamins in Infancy 94
Notes 95
Choline and Anemia 95
Alloxan Diabetes and Vitamins 95
Adequacy of Diet and Speed of Rachitogenesis 96
Oxford Nutrition Survey 96
Compounds Formed from Molds 96

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FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

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NUTRITION REVIEWS

VOL. 6 MARCH, 1948 No. 3

NUTRITION AND OPHTHALMOLOGY

An evaluation of the role of nutrition in present day ophthalmology is clearly contingent upon one's point of view. From the standpoint of the nutritional scientist good vision depends in part upon receiving certain foods, from that of the practicing ophthalmologist, poor vision in this country can seldom be accounted for solely on the basis of a nutritional deficiency.

Let us examine the role of nutrition in ophthalmology from the point of view of both the nutritionist and the ophthalmologist by recalling how diet affects vision. One approach is to consider of what the visual process consists. In simplest terms it consists of transmitting and focusing light waves and the eventual transformation of these into nerve impulses which in turn are transmitted to the brain to be interpreted. The cornea, lens, and intraocular fluids are concerned with the transmitting and focusing of light, the retina with converting it to nervous energy, and the optic nerve with transmitting this energy to the brain.

The transmission of light through the cornea is frequently impaired through changes which result in clouding and also from growth of new blood vessels. These disturbances, both of which occur clinically from a variety of causes, have been shown experimentally to result from a deficiency of riboflavin, tryptophane, lysine, or methionine. Opacity of the cornea also occurs from lack of vitamin A, and in some species excessive intake of cholesterol. The lens too depends on adequate supplies of protein and riboflavin for maintenance of clarity, and rapidly becomes cataractous following administration of galactose, lactose, or xylose. Except in the diabetic patient its focusing capacity has not been related to intake of any particular dietary constituent. The epithelium of the cornea and the lens itself contain high concentrations of ascorbic acid though no evidences of opacity occur characteristically in patients with scurvy. The clarity of the intraocular fluids does not appear to be affected primarily by nutrition but there has been a considerable amount of experimental work with animals demonstrating that deficiencies of vitamin A during gestation frequently induce physical defects in eye tissue including growth of mesodermal tissue in the vitreous humor. There is no adequate evidence of such a relationship in human experience. So much for the effects of diet on the structures affecting transmission of light to the retina.

Adequate supplies of vitamin A are essential for the transformation of light stimulus into nerve impulses, the effects of deficiency, in some instances, manifesting themselves as night blindness. It is of incidental interest that an Egyptian papyrus dated 1500 B.C. records the use of liver in the treatment of this disease. Nevertheless, moderate deficiency of vitamin A plays little role in visual function under ordinary conditions of illumination.

Transmission of the nerve impulse appears to depend upon adequate supplies of thiamine as indicated by reports of improvement in conduction defects and retrobulbar neuritis with beriberi following thiamine therapy. The cerebral process by which impulses set up by the retina are integrated into visual patterns does not appear to be related to any of the known nutritional deficiencies.

Thus, normal vision has been associated with certain specific nutrients and to this extent nutrition is an important consideration in ophthalmology. From a practical standpoint, one might profitably inquire what proportion of the cases seen by ophthalmologists in this country are of nutritional origin. Accordingly, a questionnaire

NOTICE

5 *Year*
CUMULATIVE INDEX
to
NUTRITION REVIEWS

The Nutrition Foundation believes that the material covered in NUTRITION REVIEWS during the past five years can be made more readily available through a cumulative index. Accordingly, the Editorial Office is now preparing such an index. New entries which have not appeared heretofore in the annual indexes will be included to ensure more thorough coverage. There will also be a cumulative author index covering the five year period.

The Indexes should be ready for distribution in April, 1948.

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was sent to twenty-seven members of the staff of the Massachusetts Eye and Ear Infirmary in Boston asking the number of patients seen during the past year in which ocular signs were considered to be caused by or associated with improper diet. In all, these physicians examined in their offices and in the clinic at least 30,000 patients. Ten ophthalmologists replied that they had seen no patients in which the ocular signs were, so far as they knew, related to diet. The others listed approximately 40 cases in which nutrition was believed to play some role. Most of these cases were either rosacea keratitis or toxic amblyopia. Two cases of keratomalacia were reported. It is important to note that while most of the ophthalmologists listed cases of rosacea keratitis, frequently they expressed doubt regarding whether this disease was caused primarily by a "vitamin B deficiency." Some, on the other hand, thought that other forms of keratitis and some cases of cataract might have been due at least in part, to poor diet. Thus, in the opinion of the practicing ophthalmologists in one American city, only 0.1 to 0.2 per cent of the ocular disorders seem to be related to poor diet. In view of the well established relation between various foods and ocular abnormalities this finding suggests that frank nutritional deficiencies in the population in the area under consideration are relatively rare. The greater frequency of eye diseases of apparent nutritional origin in other parts of the world suggests that here inadequate nutrition may be a causative factor of paramount importance in ocular disease. There are of course many ocular diseases of unknown etiology and it is possible that some of these may prove to be of nutritional origin. Additional experimentation by both the nutritionist and the ophthalmologist designed to obtain information concerning such possibilities represents another approach to the problem of further lessening the frequency of ophthalmologic disorders.

V. EVERETT KINSEY
Howe Laboratory of Ophthalmology
Harvard Medical School
Boston, Massachusetts

IRON COMPOUNDS FOR ENRICHMENT OF BREAD

In the enrichment program, iron has been added to flour at the mill or added to the ingredients of bread at the bakery. In either case, the selected source of iron is satisfactory only if it is harmless, assimilable, and without deleterious action on flour or bread. Ferric phytate and sodium iron (ferric) pyrophosphate have been used in mill enrichment of flour but both have been largely replaced by reduced iron. Ferric orthophosphate is generally used when enrichment is carried out at the bakery. Although the question of assimilability of compounds of iron has received a great deal of attention, the experience with iron phytate emphasizes the necessity of even more extensive investigations before any substance is employed as a source of iron in the enrichment of food (*Nutrition Reviews* **1,** *59, 269 (1942–1943);* **2,** *45 (1944)*). The net absorption of iron from the intestine and its utilization in the production of hemoglobin in man and in experimental animals have been the subjects of previous reviews (*Ibid.* **4,** *227, 291 (1946);* **5,** *45 (1947)*).

Because of contradictory reports regarding the availability of ferric orthophosphate iron, H. Blumberg and A. Arnold (*J. Nutrition* **34,** *373 (1947)*) have extended their earlier studies and have reported on the comparative values of this compound and of ferrous sulfate and ferric chloride. Their experimental procedure consisted of determinations of the rate of regeneration of hemoglobin in anemic rats fed diets consisting mainly of bread enriched with these three iron salts. The diet contained the following: dried bread 82; casein 12; iron-free salt

mixture 3; corn oil 3; and, supplemental vitamins. Casein was used to ensure the adequacy of the dietary protein. Bread was prepared in four lots, one containing no added iron and the other three lots containing ferric orthophosphate, ferrous sulfate, and ferric chloride, respectively. Suitable iron levels for each compound were obtained by the use of mixtures of the dried, powdered, negative control and enriched breads. The bread mixtures were tested in young rats that had become anemic (2.5 to 5.0 g. per cent of hemoglobin) during a five week period on a diet of cow's milk supplemented with copper and manganese. Hemoglobin determinations were made weekly for four to nine weeks. An interpolated value for one and a half weeks appeared to be the most sensitive point for comparisons and, with minor exceptions, indicated the general trend of the results of later determinations. Under the conditions of these experiments, ferrous sulfate and ferric chloride were of equal biologic availability and each was from four to five times as effective as ferric orthophosphate.

The similarity in the availability of ferrous sulfate and ferric chloride in the rat (*Nutrition Reviews* **1,** *269* (*1943*)) and in the dog (*Ibid.* **3,** *110* (*1945*)) has been reported previously. These species appear to utilize the ferric iron more efficiently than man (*Ibid.* **1,** *154* (*1943*)). The comparatively low availability of the ferric orthophosphate iron in the experiments of Blumberg and Arnold on rats throws doubt on the advisability of the use of this compound in the bakery enrichment of bread. There is no certainty that similar results would be found in man but this appears probable from the suggestive clinical findings of C. V. Moore, W. R. Arrowsmith, J. Welch, and V. Minnich (*J. Clin. Invest.* **18,** *553* (*1939*)). Blumberg and Arnold properly emphasize the need for additional studies in man and animals if the use of the iron orthophosphate is to be continued. In the meantime, it would appear wiser to use ferrous sulfate or a similar form of reduced iron as an enrichment material because of the known efficacy of this type of iron in man.

A COMPARISON OF OMNIVOROUS AND VEGETARIAN DIETS

As the population of the world increases, the necessity to use available food supplies more efficiently and to create new food sources becomes greater. Increased utilization of plant foods for human consumption and increased plantings of crops with higher yields of food materials per acre are routes through which more efficient use of available fertile land could be made. If the proportion of plant foods to be used for human consumption is to be increased appreciably, many experimental investigations must be made of the nutritional value of individual plant foods, how they can best be used to supplement each other, whether or not completely vegetarian diets are of comparable nutritional value to omnivorous ones, and if vegetarian diets are not of equal value to omnivorous ones, what are their deficiencies and how can these deficiencies best be satisfied in terms of food efficiency.

Despite the frequently expressed opinions that vegetarian diets promote health and longevity in human beings, there has been surprisingly little scientific investigation to test the validity of this statement. J. R. Slonaker (*Leland Stanford Junior Publication, University Series, no.* **9** (*1912*)) studied the effect on rats of a diet designated as strictly vegetarian but which included such foods as bread and cookies made with small amounts of foods of animal origin. The rate of growth of rats maintained on this diet was much slower and their average length of life about one-half as long as the control rats which received the same diet supplemented with meat and other foods of animal origin. Other comparisons have

been made on the effects upon rats of purely vegetarian and omnivorous diets by C.-Y. Chang, H. Wu, and T.-T. Chen (*Chinese J. Physiol.* **16,** *229* (*1941*)). The rate of growth of rats on their economically best vegetarian diets was inferior to growth on their omnivorous diets but the vegetarian rats lived as long or longer than the omnivorous rats. The male vegetarians did not live significantly longer than the omnivorous males but the female rats fed the vegetarian diet lived significantly longer than the females fed the omnivorous diet. Usual findings indicate that female rats on any dietary regimen live longer than males on the same regimen, but in these investigations the females fed the omnivorous diet did not live longer than the males fed the same diet. Since the omnivorous females did not live longer than the omnivorous males, possibly instead of the life span of the females having been lengthened by a vegetarian diet, the life span of the females on the omnivorous diet was somehow shortened.

A. J. Carlson and F. Hoelzel (*J. Nutrition* **34,** *81* (*1947*)) have described two experiments in which the comparative effects of an omnivorous diet and two purely vegetarian diets on the growth and longevity of rats were studied. The omnivorous ration used in both experiments was composed of dried "whole veal," veal bone meal, corn starch, dried brewers' yeast, an inorganic salt mixture, and cod liver oil. The vegetarian diet used in the first experiment consisted of whole wheat flour, peanut flour, wheat gluten flour, Lima bean flour, linseed meal, corn gluten meal, alfalfa leaf meal, brewers' yeast, and sodium chloride. The omnivorous diet was modified by the addition of 10 per cent cellulosic bulk-formers. In the second experiment, the vegetarian rats were allowed free selection from individual containers of whole corn kernels, whole wheat grain, pearled barley, rolled oats, sunflower seeds, peanuts, green peas, soybeans, defatted corn germ meal, defatted wheat germ meal, brewers' yeast, alfalfa leaf meal, and sodium chloride. All three rations were supplemented with lettuce ad libitum.

In the first experiment, 45 rats were fed the basic omnivorous diet and 46 rats were fed the purely vegetarian diet. Some members of each group were starved intermittently by the procedure described previously by Carlson and Hoelzel (*Nutrition Reviews* **4,** *218* (*1946*)). An additional 46 rats were maintained on the omnivorous diet with 10 per cent bulk-formers added. Detailed data from the latter group were not presented in this paper. The rats fed the omnivorous diet ad libitum attained weights 30 to 35 per cent higher than the weights of their parents at similar ages. On the contrary, rats fed the vegetarian diet weighed less than the average weights of the parent stock. The average weight attained by the males and females fed the omnivorous diet ad libitum was 591 g. and 456 g. respectively, in comparison with 354 g. and 236 g. for males and females, respectively, which were fed the vegetarian diet ad libitum. The males and females fed the omnivorous diet ad libitum lived an average of 633 and 659 days, respectively, while the males and females fed the vegetarian diet ad libitum lived an average of 595 and 649 days, respectively.

The weights attained by the intermittently fasted rats were inversely proportional to the amount of fasting, but the size of the rats, as indicated by femoral length at death, was more uniform than their weight and was nearly independent of the degree of fasting. The ages attained were, on the average, slightly higher for rats fed the omnivorous diet than those fed the vegetarian diet and greater in partially fasted rats than in rats fed ad libitum.

In the second experiment, efforts were made to use rats which were more uniform genetically than the rats in the first investigation. Five litters were started on the omnivorous diet; 11 litters were started on the omnivorous diet plus added bulk-formers. Only 2 litters had been started on

the self-selection vegetarian diet when a serious inability of these rats to exist on this diet was observed. Therefore, 7 of the 13 original vegetarians were transferred to the omnivorous diet before they were 200 days of age and the remaining 6 transferred to the omnivorous diet before they became 345 days old. The omnivorous rats in this experiment weighed more at 200 days than the corresponding rats in the first experiment; the 6 rats in this experiment which did best on the vegetarian diet weighed more at 200 days of age than comparable animals fed the vegetarian diet in the first experiment. The data indicated that the rats fed the vegetarian diet early in life and the 10 rats which were changed from the omnivorous to the vegetarian ration later in life did not grow as well nor live as long as the rats fed the omnivorous ration throughout life. The rats which were fed the omnivorous diet plus bulk-formers did not grow as well as those which received no bulk but did grow considerably better than those which received the vegetarian diet. The data on longevity of the animals with added bulk in the diet were not presented but were deferred to a later report.

Carlson and Hoelzel attributed the higher rate of growth of rats maintained on the omnivorous diet to its high content of animal protein and fat and to its high energy value and low amount of roughage. Growth studies indicated that less than half of the lower weight increases in rats on the vegetarian diets could be accounted for by their bulkiness or their non-nutritive fraction. The poorer rates of growth in rats on the vegetarian diets seemed attributable to the unsatisfactory nutritive value of the components of the diet.

Great variations in the life spans of the rats, particularly in the rats fed the vegetarian diet in the first experiment raised the question of the statistical significance of the greater average length of the life of the omnivorous rats. Analysis of the data on litter mate males alone or litter mate females alone on either ad libitum or intermittent fasting regimens did not indicate a significant difference; however, when the data from these four groups were combined, statistical analysis showed that the animals fed the omnivorous diet lived significantly longer than those fed the vegetarian diet ($P < 5$ per cent). The great variations in life expectancy among vegetarian rats are of special interest; for example, 4 vegetarian rats lived to over 1000 days of age while only one omnivorous rat lived beyond 1000 days. In addition, rats fed the vegetarian diet did not develop skin lesions nor alopecia as did most of the old omnivorous rats. Mammary tumors were larger and more frequent in the rats fed the omnivorous diet than in those fed the vegetarian diets.

In a study of this type, the question may well be raised as to what are adequate criteria for judging an abundant life and physical and mental well-being. Increasing longevity in mankind in many ways would be a burden upon civilization unless concomitantly health and mental and industrial productivity can be achieved in the added years. One cannot help wondering from an investigation of this type what were the causes of death in the two groups of animals. Since the omnivorous rats developed skin lesions and alopecia and the vegetarian rats did not, most investigators will wonder how these and other less obvious lesions affected the over-all health of the animals. Tests of achievement and learning ability possibly might supplement growth and longevity data in experiments of this general type.

As a strictly nutritional problem, many questions are raised as to the essential differences between the omnivorous and vegetarian rations which resulted in the observed differences. Carlson and Hoelzel commented on the differing fat and protein and bulk contents of the diets and studied the effect of the bulk content on the rate of growth. It would be valuable to know if supplementation of the vegetarian diet

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with fat or protein or with the specific amino acids needed to render the plant proteins more nearly complete would increase the average longevity of rats fed the vegetarian diets without increasing the incidence of skin lesions and alopecia.

ORGANIC ELEMENTS IN MATURE DENTAL ENAMEL

R. F. Sognnaes (*Harvard Dental Alumni Bull.* **7,** *3 (Nov.) (1947)*) has compiled a concise history of our knowledge of the structure of dental enamel with special reference to new advances in the investigation of the organic components in this most highly calcified tissue in the body. Since the organic elements of enamel are so highly mineralized, it is suggested that ordinary fixatives do not reach them prior to decalcification. In ordinary histologic procedures, the acids used to decalcify the hard dental structures react with the carbonates of the enamel to form carbon dioxide. When the bubbles of carbon dioxide form in the decalcification process, they have a tendency to tear the delicate and poorly fixed organic fibers and to lift the surface cuticle from its attachments to the prism sheaths and the latter from the dentin. Handling of the specimen during washing and dehydration results in the complete loss of the remainder of the organic material. These technical difficulties were sufficiently real that A. Hopewell-Smith (*New Zealand Dental J.* **27,** *54 (1931)*) concluded "everything indicates the terminative exactitude of the assertion that the enamel is a solid, dense adamantive, inorganic mass." Such viewpoints have led to the prevalent belief that mature human enamel could have no metabolic activity.

To overcome the difficulties in decalcifying enamel, it is necessary to (1) immobilize the specimen, (2) denature the protein components as they are exposed, and (3) slow down the decalcification by weak acid, low temperature, or high pressure to decrease the formation or increase the solubility of carbon dioxide sufficiently to prevent its destructive action. Under such combined circumstances, Sognnaes has been able to obtain thin paraffin sections of the organic framework of fully formed enamel. Photographs in the article demonstrated some of the characteristics of this organic component. Interestingly enough, several characteristics of enamel, such as the birth or neonatal line and the striae of Retzius, which often have been attributed to the calcification of the enamel, are of organic nature.

The broad importance of this fundamental investigation on the histology of the enamel becomes evident when one considers that a lesion of the enamel is "a stage in the development of more widespread disease, e.g., pulpitis, cysts, abscesses, and further local and focal infections." The function of these organic elements in the pathogenesis of dental disease is practically unknown. Evidence that enamel has a low metabolic activity of a type has been presented in the review by W. D. Armstrong (*Ann. Rev. Biochem.* **11,** *441 (1942)*). The fact that an organic component of definite pattern and organization can be recovered from enamel gives real promise that the mechanism and potentialities of the metabolic activity of enamel can be effectively investigated. One of the most fundamental problems in biochemistry and nutrition specifically concerns the intimate composition of the organic matrix of enamel, its effect on susceptibility and reaction to tooth decay, and ways in which its development and composition might be influenced to increase the resistance of the tooth to decay.

DISTURBANCE IN TYROSINE METABOLISM IN PERNICIOUS ANEMIA

In 1926 E. Becher, S. Litzner, and W. Täglich (*Z. f. klin. Med.* **104,** *182 (1926)*) observed that the blood phenol levels are high in pernicious anemia. Subsequently M.

Volterra (*Schweiz. med. Wchnschr.* **69,** *627 (1939)*) noted that the urinary levels of the volatile phenols are lower in relapse than in remission in this disease. M. E. Swendseid, I. F. Burton, and F. H. Bethell (*Proc. Soc. Exp. Biol. Med.* **52,** *202 (1943)*) noted that urinary excretion of total phenolic compounds is increased in untreated cases of this disease. Finally, B. M. Jacobson and Y. SubbaRow (*J. Clin. Invest.* **16,** *573 (1937)*) while studying the nature of the antipernicious anemia factor reported that l-tyrosine increases the therapeutic activity of the substance they had obtained. These observations together with the fact that tyrosine is the presumed precursor of melanin pigment and that pigmentary changes are frequently observed in pernicious anemia (early graying of the hair, vitiligo) led M. E. Swendseid, B. Wandruff, and F. H. Bethell to study tyrosine metabolism in this disease (*J. Lab. Clin. Med.* **32,** *1242 (1947)*).

Seven patients with this disease were examined before and after antianemia therapy. In every patient except one the excretion of total phenols was greater in relapse than in the remission state. A decrease in the excretion of the phenols occurred, furthermore, within forty-eight hours after the institution of liver extract or desiccated stomach therapy and preceded any changes in the corpuscular elements of the blood, including reticulocytosis. It was clear by comparison with observations in normal subjects that excretion of total phenolic compounds and hence of hydroxyphenyl acids is increased in cases of pernicious anemia in relapse.

These observations are interpreted as indicating that substances concerned with erythrocyte maturation participate directly or indirectly in the oxidation of phenolic compounds and hence of the amino acid tyrosine beyond the aromatic acid stage. Three of the known vitamins have been shown to affect tyrosine metabolism in the various biologic systems; namely ascorbic acid, pyridoxine, and pteroylglutamic acid. These vitamins, however, are either absent or present in small quantities in purified liver extracts. It does not seem possible, therefore, that the effect of liver extract on phenol excretion in pernicious anemia can be ascribed to its vitamin content. It is suggested that the antipernicious anemia principle in liver extract may activate one or more of these vitamins in some metabolic reaction concerning tyrosine. The question is raised whether some phenolic derivative may directly participate in erythropoiesis, the liver principle affecting red cell maturation by virtue of its action in the tyrosine metabolic cycle to produce this derivative.

This study represents more evidence that the metabolic disturbance associated with the clinical picture of pernicious anemia is a complex one which becomes even more intriguing as more knowledge is gained.

A CHICK GROWTH FACTOR IN COW MANURE

D. Whitson, H. W. Titus, and H. R. Bird (*Poultry Sci.* **25,** *52 (1946)*) reported the results of an experiment in which laying hens were fed diets consisting of corn, heated soybean oil meal, and alfalfa leaf meal supplemented with vitamin and mineral concentrates for forty weeks. As the level of soybean oil meal was increased from 0 to 40 per cent in increments of 10 per cent, the hatchability of the eggs produced decreased significantly regardless of the presence of adequate amounts of the essential nutrients known to affect hatchability. These high levels of soybean oil meal did not have an adverse effect on egg production, body weight, or egg size. In another experiment where the diet contained 67 per cent of either wheat or corn and 20 per cent soybean oil meal, two different samples of wheat permitted significantly higher hatchability than

did corn. Decreased hatchability was observed throughout the winter months.

These authors demonstrated that the inclusion of 8 per cent of dried cow manure possessing high androgenic potency in a good, practical, all mash diet resulted in a significant reduction in egg production (*Poultry Sci.* **25,** *143* (*1946*)). The inclusion of cow manure in which androgenic potency had been destroyed by drying at 80°C. for twenty-four hours exerted no effect on egg production. This practical ration supported high hatchability regardless of whether it was supplemented by cow manure or not. However, the inclusion of 8 per cent cow manure dried at 80°C. for twenty-four hours in a diet high in soybean oil meal and containing no animal protein significantly improved hatchability and largely eliminated seasonal variations in hatchability which were characteristic of the basal soybean oil meal diet.

Early studies of the relation of cow manure supplements to the growth of chicks fed soybean oil meal rations were made by M. Rubin and H. R. Bird (*Nutrition Reviews* **4,** *330* (*1946*)). The basal diet was complete in all of the chemically characterized vitamins (D. Whitson, J. C. Hammond, H. W. Titus, and H. R. Bird, *Poultry Sci.* **24,** *408* (*1945*)). The growth factor in cow manure was believed to be dissimilar from other postulated growth factors and was moderately soluble in water, was largely precipitated from solution at pH 3.0, did not dialyze through cellophane, and was stable toward heat. This growth factor could be transmitted from the hen through the egg to the chick. H. R. Bird, M. Rubin, and A. C. Groschke (*J. Nutrition* **33,** *319* (*1947*); *Nutrition Reviews* **5,** *237* (*1947*)) presented evidence which suggested genetic variations in the ability to transmit this growth factor to the egg.

M. Rubin and H. R. Bird (*J. Nutrition* **34,** *233* (*1947*)) have presented additional studies on the relation of the quantity and quality of soybean oil meal in all plant protein diets to the growth factor in cow manure. The effect of four levels of soybean oil meal (25, 35, 50, and 70 per cent) on the rate of growth of chicks was tested. The same procedures used in earlier experiments were employed. The ration containing 35 per cent soybean oil meal resulted in slightly superior growth to the ration containing 25 per cent soybean oil meal. Marked inhibitions of growth were observed when 50 or 70 per cent levels of soybean oil meal were fed despite the increased levels of the essential amino acids. As demonstrated previously, addition of 0.075 per cent of the acid precipitate of water extract of dried cow manure to the 35 per cent soybean oil meal diet resulted in an excellent growth stimulus. When the diets containing 50 or 70 per cent soybean oil meal were supplemented with the same amount of the acid precipitate, the resulting growth rate was as good or better than when the 35 per cent soybean oil meal diet was supplemented with cow manure or a concentrate of cow manure. The effect of supplements of 0.2 and 0.4 per cent dl-methionine to the 70 per cent soybean oil meal diet was tested. Little or no growth stimulating effect was observed. In all experiments where there was a high soybean oil meal content, there was a high mortality which was reduced by the factor in cow manure.

An experiment to test the effect of the maternal diet on the growth of chicks fed the high soybean oil meal diet compared a maternal diet which was deficient in the cow manure factor with one which contained the factor. The diet containing 70 per cent soybean oil meal resulted in a moderately reduced growth rate of the chicks whose dams had been fed the latter ration; these chicks grew much faster than the chicks fed the 35 per cent soybean oil meal diet but whose dams had received the diet deficient in the cow manure factor.

The next series of experiments was performed to test the ability of the cow manure growth factor to overcome the deficiency of

raw soybean oil meal at a level of 35 per cent, in comparison with the effectiveness of either methionine supplementation or hydrolysis of the soybean oil meal. Two samples of raw soybean oil meal were tested in the basal diet with the expected low growth. The addition of 0.2 per cent dl-methionine to the raw soybean oil meal diet resulted in rates of growth comparable to those where heated soybean oil meal was used. Supplements of the acid precipitate of cow manure improved the diets containing raw soybean oil meal but not as much as methionine additions even when high levels of the cow manure concentrate were incorporated in the ration.

The effect of enzymatic hydrolysis of the proteins in raw soybean flour was studied in one experiment by comparing the rates of growth when raw soybean flour or soybean flour hydrolysate was substituted for heated soybean oil meal at 35 per cent in the basal ration. The enzymatic hydrolysis of raw soybean flour improved its nutritional properties so that the rate of growth observed on the diet containing the hydrolysate was higher than on the diet with raw flour, but not quite as high as on the diet with heated soybean oil meal. Insufficient raw soybean flour or hydrolysate was available to test the effect of cow manure supplements.

These studies clearly indicate a definite parallelism between the effect of increasing levels of soybean oil meal in the diet on embryonic development and on the rate of growth after hatching. Supplements of dried cow manure which would improve the hatchability of eggs laid by hens fed the all plant protein diet likewise would improve the rate of growth of chicks fed the same diet. Cow manure was an effective supplement even when the soybean oil meal content of the ration was increased to 70 per cent. Bird and co-workers (*loc. cit.*) demonstrated previously that dl-methionine was at best only a partial substitute for the growth factor in cow manure when the soybean oil meal in these diets had been heat treated. This observation was substantiated in the current studies indicating that heated soybean oil meal contains barely adequate quantities of methionine but that cow manure is a more effective supplement than dl-methionine. The growth factor in cow manure on the other hand is incapable of supplementing diets containing raw soybean oil meal as effectively as the addition of dl-methionine. These observations strengthen the belief that there is some material in cow manure which is required by the chick for normal growth and development to overcome the effect of heated soybean oil meal in the diet.

The studies are valuable at the present time because of the continued insufficient supplies of animal protein concentrates used extensively by poultry raisers prior to wartime restrictions. In addition development of adequate rations for poultry comprised entirely of plant products would result in a more efficient use of basic food production. At the present time, the relation of low hatchability of eggs produced by hens fed soybean diets and of the poor growth of chicks fed the same diets to the low reproductive rate and high incidence of embryonic deformities in pigs and to reproductive failure in rats fed similar diets is rather obscure. In both the pig and the rat, increase of the alfalfa leaf meal in the ration from 5 to 15 per cent resulted in a decrease in reproductive failure (*Nutrition Reviews* **1,** *49 (1943)*). Evidence that cow manure may be a helpful supplement to the diet of hogs has been presented (*Ibid.* **3,** *79 (1945)*).

COBALT STUDIES WITH THE RABBIT

In earlier studies with the white rat, E. J. Underwood and C. A. Elvehjem (*J. Biol. Chem.* **124,** *419 (1938)*) were unable to produce evidence of a cobalt deficiency when

as little as 0.6 microgram of cobalt was ingested daily per rat. Later A. E. H. Houk, A. W. Thomas, and H. C. Sherman (see *Nutrition Reviews* **4,** *336 (1946)*) found no evidence of cobalt deficiency in the rat when they fed a basal ration which supplied only 0.03 microgram of cobalt per rat per day. J. F. Thompson and G. H. Ellis (*J. Nutrition* **34,** *121 (1947)*) fed rabbits a diet composed of whole milk collected at milking into Pyrex jars and corn grown in a low cobalt area. In the ratio of these two foods consumed by the rabbits, the cobalt content of the diet on a dry weight basis was 0.0024 parts per million. From birth to 4 weeks of age, the rabbits were not allowed access to the dam's ration. At 4 weeks of age, they were divided into groups at random; 15 of the rabbits received no cobalt supplement while 5 received an additional 4 micrograms of cobalt per day. Five animals in the first group and all 5 in the second group were given supplements of iron, copper, and manganese while the remaining 10 were given a supplement of manganese only. After 14 weeks of age, these animals also were given supplements of iron and copper. The average hemoglobin values from this point to the end of the experiment were 12.9 g. per 100 ml. for the group receiving cobalt and 12.6 g. per 100 ml. for the group not receiving cobalt. The rate of growth in both groups was fairly good but the mortality rate during the experiment was high with only 10 survivors at the end of the forty-six week experimental period. The rate of growth was slightly more rapid and the mortality rate slightly lower in the cobalt supplemented group. The consumption of corn and of milk was slightly higher for the rabbits receiving the cobalt supplements. None of these differences was sufficiently great to be of statistical significance. At autopsy of the rabbits which survived the entire experimental period, the livers of the cobalt-supplemented rabbits were found to contain 0.140 microgram of cobalt per gram in contrast to 0.014 microgram in the livers of those rabbits with no cobalt supplement. All animals appeared normal with the exception that the livers of nearly all animals in both groups were cirrhotic. This finding along with the high mortality rate and the inability of females to raise their young indicated the gross inadequacy of the diet in other respects than its iron, copper, manganese, and cobalt contents. Supplemental feeding of sodium chloride, thiamine, and cellulose had been demonstrated to be ineffective. It is unknown whether the other nutritional deficiencies of this diet could have influenced the production of a cobalt deficiency in these animals. So far as these experimental data go, they substantiate the present evidence that there is a difference in the cobalt requirement of ruminating and nonruminating animals.

On the basis of evidence described by R. A. McCance and E. M. Widdowson (*Ann. Rev. Biochem.* **13,** *315 (1944)*) that sheep suffering from cobalt deficiency were cured when cobalt was given orally but were not cured when cobalt was injected, Thompson and Ellis have presented the interesting postulate that cobalt may be essential for some of the microorganisms in the rumen which are concerned with the synthesis of "B-vitamins." The latter investigators drew attention to certain similarities between cobalt deficiency in sheep and cattle and experimental deficiencies of some members of the vitamin B-complex: anorexia, impaired reproduction, and hypochromic microcytic anemia. While none of these deficiency signs is specific to any particular deficiency, the similarity is an interesting one, especially since J. F. Filmer (*Australian Vet. J.* **9,** *163 (1933)*) demonstrated that liver was effective in curing endemic "coast disease" and that liver ash was ineffective.

DIETARY OXALATES, PENICILLIN, AND EXPERIMENTAL TOOTH DECAY

The destructive effect on the enamel and dentin of the regular consumption by experimental animals of acid beverages in place of drinking water has been described (*Nutrition Reviews* **4,** *17* (*1946*)). The weights of the lower molar teeth were reduced in some instances by as much as 35 or 40 per cent. Addition of sucrose to the acid solutions appeared to increase their decalcifying properties. Inclusion of 1 to 20 parts per million of fluorine as soluble fluorides in the acid-sucrose beverages resulted in partial protection of the tooth surfaces from the decalcification process. Later R. A. Gortner, C. M. McCay, J. S. Restarski, and C. A. Schlack (*J. Nutrition* **32,** *121* (*1946*)) reported that ingestion by white rats of small amounts of soluble oxalates in the diet or in the drinking water resulted in a hard deposit on the teeth which was "presumably calcium oxalate" and which grossly resembled human dental calculus. Oxalic acid solutions having a pH as low as 2.1 administered for one or two week periods did not etch the enamel of rats *in vivo*, unlike citric, lactic, phosphoric, and sulfuric acids at the same or higher pH values. When oxalic acid or sodium oxalate was present in the food or drink, the decalcification, which ordinarily accompanies the ingestion of 0.005 per cent phosphoric acid or 0.20 per cent citric acid in 10 per cent sucrose solution, was greatly decreased or prevented. When natural oxalate-containing foodstuffs such as spinach and rhubarb were incorporated into the diet, the characteristic deposits on the molar teeth were visible within a week. The data from these short-term experiments indicated that oxalates were more effective than fluorides at practicable levels in preventing *in vivo* dissolution of enamel by acid-sucrose solutions.

The significance of these data is unknown in terms of human calculus or of enamel erosion and decalcification or of the initiation and progress of carious lesions. A possible correlation between oxalate ingestion and dental caries incidence in man has been suggested by the investigations of M. R. Jones, N. P. Larsen, and G. P. Pritchard (*Dental Cosmos* **72,** *685* (*1930*)) who observed a lower incidence of dental caries in Hawaiian children fed diets consisting primarily of taro (poi) and fish than in those children fed diets high in refined carbohydrates. Taro contains about 0.49 per cent free and combined oxalic acid (A. L. Winton and K. B. Winton, "The Structure and Composition of Foods," *John Wiley and Sons, New York* (*1935*)). N. P. Larsen (*Plantation Health* **11,** *19* (*Oct.*) (*1947*)) presented additional data on the relation of natural foodstuffs, in particular taro, to the incidence of tooth decay in Hawaiian children and in recently immigrated Filipino children. C. A. Schlack and co-workers (*J. Dental Research* **26,** *383* (*1947*)) studied the effect of oxalate ingestion on the susceptibility of white rats to tooth decay. One hundred and sixty-five weanling white rats were divided into five major experimental groups. In each experiment, the rats were distributed into three experimental treatments according to litters. In all experiments, they were fed a coarse corn meal caries-inducing ration for one hundred days with various additions of oxalates to the diet or to the drinking water. The effect of additions of 0.02, 0.06, 0.11, and 0.33 per cent of sodium oxalate to the ration was studied in the first two experiments. In two of the other experiments, the effect of the incorporation of 0.0002, 0.002, 0.02, and 0.22 per cent of potassium oxalate into the drinking water was tested. No reduction in the incidence nor in the extent of tooth decay was observed when any of these amounts of oxalate were ingested in the ration or in the drinking water. In the fifth experiment, additions of 1.6 and 8 per cent of dehydrated spinach as a source of oxalates in their nor-

mal occurrence were made to the coarse corn meal ration. Again no reduction in the incidence nor in the extent of tooth decay was observed.

Under the conditions of these experiments, the addition of oxalates to the ration or to the drinking water was ineffective in altering the initiation and progress of the carious lesions in the white rat. This is not entirely a fair test of the possible role of oxalates in maintaining normal tooth structure in man. Those carious lesions produced in white rat molars by the coarse corn meal diet have been demonstrated to be initiated largely through mechanical trauma (R. F. Sognnaes, *Am. J. Orth. Oral Surg.* **27,** *552 (1941)* and B. Kearney, R. F. Sognnaes, and B. G. Bibby, *J. Dental Research* **20,** *280 (1941)*). So far as is known, carious lesions in man are not preceded by mechanical injuries. Schlack *et al.* stated that incomplete unpublished data with the cotton rat tended to show the same results as observed in white rats. No statement was made of the dietary regimen used for the cotton rat studies. If the ration used was a purified one of the type previously described (*Nutrition Reviews* **3,** *83 (1945)*), the mechanical effects in the initiation of carious lesions would be a great deal less than in the white rat experiments and by that token the cotton rat investigations could be expected to have greater application. On the basis of the caries experiments on the white rat, and in view of the mechanical initiation of carious lesions in the white rat, these authors are not justified in their conclusion "This work tends to minimize the importance of the acidogenic factor in the initial stages of the dental caries process."

F. J. McClure and W. L. Hewitt (*J. Dental Research* **25,** *441 (1946)*) studied the effect of penicillin in the diet and drinking water of white rats on the susceptibility to tooth decay. The rats were fed a corn meal caries-inducing diet containing 75 units of the sodium salt of penicillin per gram and drank water containing 75 units per milliliter. Control litter mates received the corn meal diet but no added penicillin. After one hundred and twenty-five days on experiment, the rats were killed for caries diagnosis microscopically. Forty-one of the 82 white rats on the control regimen had carious lesions in a total of 67 teeth while none of the 82 litter mates which received penicillin had any carious lesions. The Lactobacillus acidophilus counts of the rats receiving penicillin were lower than the counts of the control animals. Penicillin did not affect the food or water intake nor the rate of growth of the rats. In this experiment again, the mechanical origin of the carious lesions must be considered. It is interesting to note that these investigators did not record the occurrence of any fractures as such in either experimental or control animals, although undoubtedly they must have occurred.

H. A. Zander and B. G. Bibby (*J. Dental Research* **26,** *365 (1947)*) studied the effect of penicillin on acid production in saliva and on the incidence of carious lesions in the hamster. When 4 ml. of saliva were incubated with 1 ml. of 20 per cent glucose solution for four hours, the pH of the mixture decreased to 4.0. When as little as 10 units of penicillin were added to a comparable saliva-glucose mixture prior to incubation, the pH of the mixture did not change. In an *in vivo* application of the above experiment, 4 subjects used as a mouth rinse 20 ml. of distilled water containing 1000 units of penicillin. Saliva was collected at half hour intervals and incubated as above with 1 ml. of 20 per cent glucose solution. The pH of the saliva samples taken immediately after the mouth rinse decreased to an average of 4.6. The saliva samples taken as early as one-half hour after the penicillin mouth rinse and as long as two hours afterwards did not decrease appreciably in pH. No counts were made of the effect on the concentration of Lactobacillus acidophilus or other acid-forming organisms. These *in vitro* and *in vivo* studies indicate that the

presence of penicillin in the amounts studied decreased the reduction of pH observed ordinarily when saliva is incubated with glucose.

Zander and Bibby fed three groups of 8 male Syrian hamsters each a caries-producing diet beginning at weaning age. The molar teeth of the hamsters in the first group were brushed once daily with a commercial dentifrice. The molar teeth of the hamsters in the second group were brushed daily with the same commercial dentifrice into which had been incorporated 1000 units of penicillin per gram. The hamsters in the third group did not have their teeth brushed. The average number of carious lesions in the three groups was: 5.87, 0.86, and 19.0, respectively. The average number of carious teeth was 3.87, 0.86, and 8.87, respectively. These data appeared to indicate that brushing of hamster teeth with a commercial dentifrice resulted in a reduction in the incidence of carious lesions and that the addition of penicillin resulted in a further reduction. It is of interest to note that the experimental period was only thirty-five days which is much lower than the approximately one hundred day period commonly employed for the study of carious lesions in the hamster (*Nutrition Reviews* **5,** *203 (1947)*), the cotton rat (*Ibid.* **3,** *83 (1945)*), and the white rat. The reason for the reduced experimental period was not mentioned as would be expected in as great a departure from accepted procedures. Though P. H. Keyes (*loc. cit.*) stated that carious lesions could be characterized histologically as early as ten days after the beginning of the experiment, the thirty-five day period seems to be too brief for infallible diagnosis of carious lesions as distinguished from various stains and mechanical injuries. In addition no mention was made as to whether the hamsters were distributed according to litter mates or at random. When the overlapping of values is noted within these small experimental groups even though the average values for caries incidence are widely different, it appears that greater care in the experimental design with a longer experimental period, litter mate distribution of animals, and larger groups of animals would have yielded data of more importance.

Zander and Bibby stated "The above findings offer further evidence that the fermentation of carbohydrates by oral bacteria is important in the etiology of dental caries." This appears to be a strong statement on the basis of the data published and in view of the many other possibilities which might also have been tested and should be tested prior to the establishment of this hypothesis. For example, possibly proteolysis was just as much inhibited by penicillin as acid formation. Thus if we postulate that the primary lesion in dental caries is in the organic matrix or in the lamellae, the reduced proteolysis in the presence of penicillin would be of greater importance than the concomitantly reduced carbohydrate fermentation. In other words correlation does not necessarily indicate causation.

INACTIVITY OF INDOLE-3-ACETIC ACID AS A "PELLAGRAGENIC" FACTOR IN THE RAT

The role of corn in the etiology of pellagra has been discussed several times in this journal (*Nutrition Reviews* **3,** *267 (1945)*; **4,** *334 (1946)*; **5,** *163 (1947)*). It is agreed that diets containing large amounts of corn are peculiarly effective in producing niacin deficiencies in animals, and that deficiencies thus produced are alleviated by ingestion of either niacin or tryptophane. The effectiveness of tryptophane appears to be due to its partial conversion to niacin in the animal body. The "pellagragenic" activity of corn may be ascribed in part to its low tryptophane content, and in part to an imbalance

in its content of other amino acids (W. A. Krehl, P. S. Sarma, L. J. Teply, and C. A. Elvehjem, *J. Nutrition* **31,** *85 (1946);* W. A. Krehl, L. M. Henderson, J. de la Huerga, and C. A. Elvehjem, *J. Biol. Chem.* **166,** *531 (1946);* A. C. Groschke and G. M. Briggs, *Ibid.* **165,** *739 (1946)*). An additional factor involved would appear to be the presence of a toxic material or antivitamin, the effect of which is specifically counteracted by niacin or by tryptophane.

Evidence for the existence of such a "pellagragenic" agent in corn was supplied by D. W. Woolley (*Nutrition Reviews* **4,** *334 (1946)*) who succeeded in concentrating the toxic material about one hundred thousand fold, but did not establish its identity. Woolley used mice as his experimental animal. Subsequently, E. Kodicek, K. J. Carpenter, and L. J. Harris (*Lancet* **II,** *491 (1946)*) reported that indole-3-acetic acid exerted the same type of "pellagragenic" activity in the rat, and since this substance is known to occur in considerable amounts in corn, postulated its identity with Woolley's "pellagragenic" factor. This observation is difficult to reconcile with a previous report of Krehl, Henderson, de la Huerga, and Elvehjem (*loc. cit.*). In investigating the ability of tryptophane to substitute for niacin in the diet of rats, these authors fed several compounds structurally related to tryptophane, including indoleacetic acid. Although this compound was added to a diet deficient in both niacin and tryptophane, neither stimulation nor further depression of growth was observed.

F. Rosen and W. A. Perlzweig (*Arch. Biochem.* **15,** *111 (1947)*) have reinvestigated the possible relationship of indoleacetic acid to niacin deficiency in rats. A synthetic-type ration was fed containing casein 10, sucrose 83, salts 4, corn oil 3, cystine 0.2, and all vitamins except niacin. Four groups of 6 animals each were placed on experimental diets as follows: (A) basal ration, (B) basal ration plus 10 mg. per cent of indoleacetic acid, (C) basal ration plus 50 mg. per cent of l-tryptophane, and (D) basal ration plus 5 mg. per cent of niacinamide. Animals of groups A and B gained respectively an average of 13.2 and 12.5 g. per rat per week over a four week period; following supplementation with tryptophane the rate of gain increased to 17 and 16 g. per rat per week over a two week period. Control rats of groups C and D gained an average of 18.6 and 16.3 g. per rat per week over the six week period. Excretion of N′-methylnicotinamide by animals in groups A and B during the initial four weeks was approximately equal, and was less than 10 per cent of that of animals of the supplemented groups C and D. In the final two weeks during which oral supplements of tryptophane were given to animals of groups A and B, excretion of N′-methylnicotinamide increased approximately thirty-seven fold in each case, again to equivalent levels. Thus ingestion of indoleacetic acid by rats in amounts over six times as great as those reported by Kodicek and co-workers to induce niacin deficiency, failed to affect growth of niacin deficient rats adversely, or to affect the efficiency of conversion of tryptophane to niacin by these animals. The authors are thus unable to confirm the "pellagragenic" effects of indoleacetic acid in rats. Similar failures to confirm this action of indoleacetic acid have been reported in abstract form by W. A. Krehl, A. Carvalho, and G. R. Cowgill (*Federation Proceedings* **6,** *413 (1947)*) and by H. P. Sarett and G. A. Goldsmith (*Ibid.* **6,** *288 (1947)*). An explanation of the great discrepancy in results of these authors and those of Kodicek and co-workers (*loc. cit.*) cannot be attempted because of the brevity of the report by the latter authors. It is evident, however, that if corn does indeed owe part of its "pellagragenic" effects in rats to a toxic factor, the chemical identity of this substance remains to be determined.

OSTEOMALACIA DUE TO INCREASED RESISTANCE TO VITAMIN D

Osteomalacia may be due to a lack of vitamin D or to alterations in the metabolism of calcium and phosphorus as a result of renal disease or hyperparathyroidism. An excellent summary of many careful studies of these forms of osteomalacia and a critical review of the literature has been presented by F. Albright and co-workers (*Medicine* **25,** *399 (1946)*). These reviewers subdivide the osteomalacia which is due to lack of vitamin D into (a) simple vitamin D lack, (b) resistance to vitamin D, and (c) steatorrhea. They further note that the simple vitamin D lack, stated to be widespread in the Orient, remains as yet undemonstrated in the United States. This is the type of osteomalacia which corresponds to simple rickets and responds rapidly to small doses of vitamin D.

The type of osteomalacia which accompanies steatorrhea is also due to lack of vitamin D and likewise responds to moderate doses of vitamin D. It is due to defective fat absorption, and is accompanied by other evidences of poor absorption of fat soluble vitamins such as hypoprothrombinemia, low concentration of carotene in the blood, low levels of vitamin E in the blood, and the like.

The osteomalacia which is due to resistance of vitamin D was clearly described in the report by F. Albright, A. M. Butler, and E. Bloomberg (*Am. J. Dis. Child.* **54,** *529 (1937)*). These workers reported the case of a 16 year old boy who had suffered from rickets since 1 year of age and had been under medical observation for fourteen years. The clinical, x-ray, and chemical findings were compatible with a diagnosis of rickets. The changes in calcium and phosphorus metabolism were similar to those observed in ordinary rickets. The child had failed to respond to doses of approximately 1100 USP units of vitamin D per day for ten years plus intensive heliotherapy. There were no evidences of defective absorption and intravenously injected calciferol in ordinary doses did not alter the metabolic defect. No evidences could be obtained of parathyroid dysfunction other than the secondary hyperparathyroidism which is characteristic of simple rickets. Massive doses of vitamin D ranging from 150,000 to 1,500,000 USP units daily by mouth corrected the disorder of metabolism and promoted healing of the rickets. It was concluded that faulty absorption of vitamin D was not a factor in this case but, rather, that the patient represented a form of rickets due to an intrinsic resistance to the action of the antirachitic vitamin—a resistance which could be broken down by massive doses.

A somewhat similar case of vitamin D resistance has now been reported by R. A. McCance (*Quart. J. Med.* **16,** *33 (1947)*). In this patient, however, the raised resistance to vitamin D appears to have been acquired at approximately 15 years of age. Prior to this age, the girl had distinguished herself at both games and athletics and at the age of 14 had won the school high jump. From the fifteenth to the seventeenth year she developed weakness of the ankles which progressed to extreme leg weakness, pain on walking, a rolling gait, and decalcification of the skeleton. During the period of the next two or three years the clinical picture deteriorated and chemical studies revealed a normal serum calcium but a low serum phosphorus level and elevated alkaline phosphatase—all findings compatible with the diagnosis of osteomalacia. X-ray photographs of the skeleton demonstrated pronounced decalcification with several spontaneous fractures or pseudofractures (Milkman's syndrome *vide infra*). There was no steatorrhea or other evidence of absorptive defects and exploration of the parathyroids detected no abnormality.

In view of the late age of onset of the dis-

ability, the reliable history of a good dietary intake, and the observed failure to respond to ordinary doses of vitamin D, the diagnosis of resistant osteomalacia was entertained. During a preliminary seven day study of mineral balance the subject was found to be in definite negative calcium, magnesium, and phosphorus balance. Five hundred thousand I.U. of vitamin D_2 were then administered daily and upon re-study of the mineral balance it was found that for all three of the above elements positive balances had been established. X-ray photographs made approximately four months after institution of therapy demonstrated considerable recalcification of the bones with callus formation at the site of the pseudofractures. There occurred a rise in inorganic phosphorus and a gradual decrease in alkaline phosphatase following treatment with the massive quantities of vitamin D. After administration of a total dose of 21,000,000 units of vitamin D, anorexia had developed and this agent was discontinued because of the possibility of toxicity—a manifestation which had developed following the large doses of vitamin D given to the case observed by Albright, Butler, and Bloomberg (*loc. cit.*). Symptomatic recovery, chemical, and radiologic improvements paralleled each other after the total dosage of 21,000,000 units had been given.

This case of "raised resistance to vitamin D" (RRD) differs from that reported by Albright *et al.* primarily in that it was acquired at a relatively late age. In a review of the literature, McCance notes that other cases of acquired RRD have been reported. This syndrome produces rachitic changes in children and progressive weakness, pain, and decalcification of the skeleton in adults. That the syndrome is rare goes without saying, and McCance cautions against thinking of therapy at the level of dosage required by these individuals as a "requirement."

The case reported by McCance exhibited so-called pseudofractures on x-ray. These are ribbon-like bands of decalcification which may occur at any site but are usually symmetrical in distribution. This roentgenologic finding was emphasized by L. A. Milkman (*Am. J. Roentgenol.* **32,** *622 (1934)*) and described as "a new skeletal disease, which is progressive, fails to respond to medication and may end fatally." Albright and co-workers (*Medicine, loc. cit.*) regard the appearance of these pseudofractures as a manifestation of osteomalacia and not as a specific disease entity. They recommend, however, that the term "Milkman's syndrome" be retained as a useful one for describing the roentgenologic picture.

These studies and re-evaluations are evidence of the progress which is being made in the exceedingly difficult field of calcium, phosphorus, and vitamin D metabolism in man. The gradual sorting and classification of osteomalacia of varying etiology are examples of the slow clarification of seemingly contradictory experiences which result from cumulative clinical experience in such fields.

FATS IN RAT NUTRITION

H. J. Deuel and co-workers (*J. Nutrition* **33,** *569 (1947)*) studied the relation of diets containing varying amounts of cottonseed oil or margarine fat to growth, reproduction, and physical capacity of white rats. The fat content of the diets varied from 0 to 50 per cent by weight and from 0 to 69.6 per cent of the total calories. Both cottonseed oil and margarine fat were tested at several fat levels. The composition of the diets was adjusted in such a way that the ratio of protein, water soluble vitamins, cellulose, and salts to calories would be identical in all diets. In ad libitum feeding, the intake of each of these components would be proportional to the caloric intake, and in iso-

caloric feeding experiments, the intake of each of these components would be the same for the animals in different dietary groups. One per cent of ethyl laurate was used as a carrier of the fat soluble vitamins in one of the fat free diets, while 1 per cent of methyl linolate replaced ethyl laurate in another fat free diet. Cottonseed oil was used as the carrier of fat soluble vitamins in the diets which contained fats. The rats used in these experiments were weaned at 21 days of age and were distributed uniformly according to litter mates among the dietary groupings. There were 25 females and 25 males in each dietary group. In the first series of experiments, these diets were fed ad libitum.

The rates of growth fell into two rather definite groups, being poorest in the animals on the "fat free" diets, and best in those with a 10 to 50 per cent fat diet and with no significant difference between cottonseed oil and margarine. Greatest growth was accompanied by highest food consumption, and the efficiency of food utilization was essentially the same on the different diets.

Breeding tests were performed with the female rats in the cottonseed oil series. Sexual maturity was reached in 8 to 9 weeks on the two fat free diets but in 6.3 to 7.0 weeks in the females consuming the various levels of fat. Eight to 10 females of each dietary group were mated after sixteen weeks on the experimental diets. Fertility was lowest in the fat free diet with methyl linolate added where no pregnancies were obtained in nine matings. In the fat free diet with no added source of unsaturated fatty acids, 2 litters were born as a result of nine matings. On all diets containing fat, the per cent of matings which were successful, the average number of animals per litter, and the average weight of individual rats at 3 days and at 21 days were higher than observed on the fat free diets. Considerable variation in fertility and in mortality among the young rats was observed in the litters born to rats receiving the diets which contained fat, and the rate of mortality was sufficiently high to cause one to suspect that there were nutritional deficiencies in these diets unrelated to the fat contents of the diets.

A description of the methods used to evaluate the physical capacity of the rats in the above experiments and the results obtained have been presented (*Nutrition Reviews* **5,** *272* (*1947*)). A regular and statistically significant increase in physical capacity was found as the fat content of the diet was increased which was unrelated to the addition to the diet of essential unsaturated fatty acids in the fats. Since preliminary studies showed a positive correlation between physical capacity and weight, and since growth was greater in rats fed diets high in fat, these authors suggested that the greater physical capacity of rats fed the high diets might be due to their larger size.

B. T. Scheer, D. F. Soule, M. Fields, and H. J. Deuel (*J. Nutrition* **33,** *583* (*1947*)) extended the above investigations by studying the effect of the fat level in the diet on the growth, mortality, and recovery in rats maintained on restricted caloric intakes. In a preliminary experiment, male rats 21 to 28 days old were used to determine the lowest caloric intake which would permit survival during a twelve week period. Stock diet was fed at levels ranging from 13 to 35 calories per day for twelve weeks. Twelve calories per day were calculated to be the minimum caloric intake for this purpose. Three hundred weanling rats were distributed with 25 males and 25 females in each of six groups. One group was fed a stock diet composed of natural foodstuffs while five groups were fed the synthetic diets containing 0, 5, 10, 20, or 40 per cent cottonseed oil, respectively. The fat free diet was supplemented with 2.5 per cent methyl linolate. Weighed portions of diets sufficient to provide 24 calories were supplied to each animal every other day.

The diets containing varying amounts of

cottonseed oil allowed slightly higher growth responses during the twelve week period than the fat free diet in both sexes. However, by far the greatest number of deaths during the restriction period occurred among the males and females fed the diets containing 5 and 10 per cent cottonseed oil. The lowest mortality was observed among the males and females fed the 40 per cent fat ration and among those rats fed the stock ration which contained only 14 per cent fat.

At the end of the caloric restriction period, the survivors in each group were allowed to consume the same diets ad libitum. The weights of the males were recorded for twelve additional weeks and those of the females for three weeks. Growth responses were greater among those animals fed the fat diets than those fed the fat free diet. After three weeks on recovery, the females were used for breeding studies. None of the females matured during the twelve week restriction periods but matured soon after the same diets were allowed ad libitum. Again fertility and lactation were more satisfactory on the diets containing fats than on the fat free diet.

B. T. Scheer, J. F. Codie, and H. J. Deuel (*J. Nutrition* **33,** *641 (1947)*) tested the effect of the fat level in diets during the maintenance of young adult rats on restricted caloric regimens. For the first eight weeks of this experiment, 24 calories were provided per day for each of 300 rats. For the next four weeks, only 12 calories were allowed each day. At the end of the experimental period, the males had lost 40 to 50 per cent and the females 30 to 40 per cent of their body weight. The effects of the same rations were studied as in the caloric restriction in weanling rats. The greatest average weight losses were observed in the animals fed the diets containing 0, 5, and 10 per cent fat and lowest in the rats fed the stock diet and the diets containing 20 and 40 per cent fat. The highest mortality rate was observed in the rats fed the diets containing 5 and 10 per cent fat and on the fat free diets. The lowest rate of mortality was observed on the stock diet, followed by the diet with 40 per cent cottonseed oil.

At the end of the twelve week period, most of the surviving animals were fed the same diets ad libitum. Recovery of body weight was most rapid among the animals receiving the stock ration or the diets containing high amounts of fats. Tests among the females indicated that recovery of reproductive capacity, especially with respect to lactation, lagged behind recovery of body weight or physical capacity.

B. T. Scheer and co-workers (*J. Nutrition* **34,** *581 (1947)*) determined the comparative composition of rats in relation to the intake of fat and calories. The fat content of the body was not closely related to the fat content of the diet; the rats which received the diet containing 20 per cent fat ad libitum had a slightly higher average fat content than those in the other groups. Caloric restriction in weanling and young adult rats resulted in relatively high water content, slightly increased ash and protein levels, and greatly reduced fat and carbohydrate. From the data presented, it appeared that the greater body size attained on the diets containing fat was not solely due to the deposition of fat and that the effects of varying fat content of the diet in resistance to undernutrition and on physical capacity were not related to differences in body composition.

These several types of experimental data demonstrate the inability of the white rat to grow, maintain itself, and reproduce on diets deficient in fats even though adequate amounts of the essential unsaturated fatty acids were supplied. The data of Deuel and co-workers do not give any suggestions as to what the role of fats might be nor as to what fraction of the fat might be active in maintaining this function. At least one growth promoting factor in fats, vaccenic acid, has been postulated in addition to the essential unsaturated fatty acids, linoleic, and arachidonic (*Nutrition Reviews* **5,** *96*

(*1947*)). Since vegetable oil diets are expected to be low in this factor (R. P. Geyer *et al.*, *J. Biol. Chem.* **169,** *227* (*1947*)), the possible role of vaccenic acid in these studies cannot be neglected; on the other hand, the different performance of the groups in such general functions as growth, reproduction, and physical capacity possibly suggests a general benefit of a certain minimum amount of fat in the diet.

NUTRITION STUDIES WITH GERM-FREE ANIMALS

Studies during recent years of the nutritional requirements and the synthetic powers of microorganisms have focused attention upon the important similarities between microorganisms and higher animals. In addition, it has been realized frequently that the action of microbes within an animal (usually in the intestinal tract) may alter the true nutritional requirements and metabolism of the host. The total extent of their influence is not known, but numerous studies related to specific nutrients have been made in which the microbial population has been reduced by sulfonamides (*Nutrition Reviews* **1,** *175* (*1943*); **3,** *35* (*1945*)), antibiotics (G. A. Emerson and D. G. Smith, *J. Pharmacol. Exp. Therap.* **85,** *336* (*1945*); D. G. Smith and H. J. Robinson, *J. Bact.* **50,** *613* (*1945*)), special diets, for example, rachitogenic (H. Friedman, *J. Nutrition* **12,** *165* (*1936*)), or cecectomy (A. Taylor, D. Pennington, and J. Thacker, *Univ. Texas Pub.* **4237,** *135* (*1942*)). The results of these indicate that deficiency symptoms may develop in rats on purified diets when the intestinal bacteria are altered in character or reduced in number. Biotin and folic acid corrected some of these deficiencies.

Although the above technics are capable of reducing the intestinal bacterial population in rats, they do not succeed in rendering animals germ-free. Moreover, it is possible that the drugs employed may impose specific changes in metabolism which would be interpreted as being of nutritional origin. Hence a satisfactory means of producing germ-free animals should be of value in nutritional studies.

L. Pasteur (*Compt. rend.* **100,** *68* (*1885*)) early raised the question whether animal life could exist in the absence of bacteria. A tentative affirmative answer was provided by G. H. F. Nuttall and H. Thierfelder (*Z. f. physiol. Chem.* **21,** *109* (*1895*); **22,** *62* (*1896*); **23,** *231* (*1897*)) with guinea pigs, which were obtained germ-free by cesarean section and maintained for thirteen days in sterile cages on autoclaved diets. Later, M. Schottelius (*Arch. Hyg.* **34,** *210* (*1899*); **42,** *48* (*1902*); **67,** *177* (*1908*)) and M. Cohendy (*Ann. Inst. Pasteur* **26,** *106* (*1912*)) working with chickens, were able to maintain them in a similar manner for a maximum of twenty-nine days. It seems probable that heat labile food factors were destroyed during sterilization of the diets and that failure of the animals to survive may have been due to the development of specific avitaminoses.

This point was recognized by G. Glimstedt (*Skand. Arch. Physiol.* **73,** *48* (*1936*)) who reinvestigated the problem using guinea pigs. He designed a wooden, aluminum lined cage which was equipped with glass viewing ports. Flexibility was provided by arm-length rubber gloves which were built into the side of the apparatus. An attendant could thus "step in" to the gloves and carry out necessary operations while viewing the system through the ports. Sterilization of the system could be accomplished and special devices, not described, were included for the introduction of food and removal of excreta.

The animals were first obtained in germ-free condition at birth by cesarean section, care being taken to maintain aseptic con-

ditions. The newborn animals were placed into metabolism cages in the germ-free system, and were fed milk through a pipet. After about two weeks on this regimen, the animals learned to drink from an automatic pipet, and shortly thereafter accepted solid food. The solid mixture was continued to the end of the experiment.

The milk diet was prepared from cow's milk, with the protein, fat, and salt content modified to simulate guinea pig milk, with an addendum of 2 per cent of crude lactalbumin. The solid diet was composed of: rolled oats 50 parts, wheat bran 20, dried milk 15, wheat germ 5, Marmite 3.5, butter 10, sodium chloride 1, salt mixture 0.5. In addition, each animal received supplements of yeast extract and vitamins C, A, and D.

The above experiment was continued for two months, but the results were disappointing. Several of the animals contracted pneumonia, and diarrhea was a common symptom. Only 19 of 59 animals survived. The statement was made that the surviving animals appeared healthy, and that aseptic conditions prevailed. Examination of the hair, skin, and alimentary tract revealed no microorganisms, and extracts of these tissues did not contaminate nutrient agar slants. In the light of present knowledge, however, it is perhaps not surprising that the guinea pigs failed to survive, for their nutritional requirements are more complex than those of the rat, and pneumonia outbreaks are more common.

J. A. Reyniers, P. C. Trexler, and R. F. Ervin (*Lobund Reports, Univ. Notre Dame* **1**, *1, 87 (1946)*) report experience with germ-free animals gained over a period of several years. They point out that maintenance of aseptic conditions is extremely difficult and for long-term experiments they have gone to great lengths to provide means for frequent sterilization of their germ-free system. The types of apparatus developed by them represent variations of T-shaped or H-shaped steam autoclaves, which have two or more compartments 32 inches in diameter and 42 inches long, each of which can be closed off from the others and sterilized. These are also equipped with glass viewing ports and arm-length neoprene gloves. Food and water are sterilized by autoclaving in the compartment next to the animals, and sterile air is led into the system after filtration through glass wool. By exercising rigid control of operational technics these workers claim that animals have been maintained in these systems without contamination for over three hundred days. The largest multiple unit in operation, which contains sixteen of the standard units above, is reported to cost over $100,000 and to require twenty or more people for maintenance. A still larger unit is also described. It is designed for occupancy by a large animal colony. Attendants dressed in special diving suits may enter through a germicide trap. Finally, a simpler germ-free unit, which is employed for short-term experiments and operations, is described. It consists of a glassed-in hood, which possesses all the features of the more elaborate system except the steam sterilization; germicides and ultraviolet light are used instead. Weights of all animals are obtained with a triple beam steel balance placed within the system.

Reyniers and co-workers have used rats in most of their studies. They report that successful cesarean delivery and rearing of these animals (about 3000 have been delivered) requires careful timing of the pregnancy. Young delivered within a few hours of term grow nearly as well when suckled by a foster mother as do normal born rats, but when the delivery is made twenty-four hours or more from term, the young do not survive. The development of a suitable diet for hand-feeding rats was apparently difficult. The average survival of rats placed on raw or condensed cow's milk was three and six-tenths days and on autoclaved cow's milk, four and five-tenths days. Cow colostrum and goat milk gave similar results. Rat milk could be used up to five days' lactation,

but after seven to fourteen days' lactation, it was also unsuitable. The routine use of rat milk would of course be impractical, moreover it could not be tolerated after sterilization.

The stomach contents of 0 to 6 day old suckling rats when diluted with water to the approximate consistency of milk, provided a satisfactory substitute for rat milk. From these and other experiments it seemed likely that the failure with cow's milk was mainly due to nonutilization of the protein. The casein finally adopted was a curd, obtained by slow precipitation of casein with hydrochloric acid. This fine flocculent precipitate was designated as R-cas. The composition of the diet per 100 ml. was as follows: R-cas 17.75 g., lactalbumin 0.6 g., salt mixture 0.8 g., raw 18 per cent cream 75.0 ml., 0.9 per cent sodium hydroxide 6.0 ml., distilled water 9.0 ml. Cream was used as the fat source because it homogenized easily. This diet was designated as 24-A. Animals which were maintained after weaning were transferred to a solid diet (S-R-5) which had the following composition: Crisco 10 per cent, polished rice 65, vitamin free casein 25, salt mixture 0.04, cod liver oil 0.02, and "B vitamins" 75 mg. per cent. With diet 24-A, about 40 per cent of over 200 young were raised to 18 days of age. Of those surviving, 87 per cent lived to 60 days. The authors felt that the losses during the early part of the experiment could be accounted for largely by accidental infection, although the data indicate that dietary factors were involved. Removal of the lactalbumin resulted in complete mortality by the third day. Alteration of the salt mixture, or the addition of whey from the R-casein or from cow colostrum did not improve the response, but the addition of ascorbic acid to the diet (diet 24-N) enabled 22 of 23 rats to survive the eighteen day experiment. Moreover, bloating symptoms which were observed on diet 24-A were largely eliminated. Animals on the ascorbic acid-free diet showed a drop in weight after birth, requiring two to four days to gain their birth weight, whereas the animals receiving ascorbic acid gained steadily. Since rat milk contains ascorbic acid, it cannot be stated whether normal suckling rats require this vitamin, but the results quoted here indicate clearly that the germ-free rat requires it during the suckling period.

Gain in weight on diet 24-N was slower among germ-free rats than in normally suckled controls; at fourteen days the germ-free animals averaged 12.1 g. compared with 23.9 g. for the controls and 8.7 g. for rats on diet 24-A. However, when 2 germ-free females of the S-R-5 group were compared with one normal female on diet S-R-5 at two-hundred and forty days, the weights were 162 and 157 g. respectively. The red blood count and hemoglobin values were virtually the same in the two series, but the white cell counts of the germ-free rats were 9600 compared with 3900 in the control. In the absence of more complete weight data, it appears likely that microorganisms play a part in improving growth during the early life of the rat.

Three germ-free females gave birth to 20 young, of which they reared 14 to weaning. After one hundred and fifteen days the young were killed. All were bacteria-free, and their weights compared favorably with normal controls. Histologic examination revealed enlarged cecums in all germ-free animals, as well as increased fat deposits about the abdomen, but underdevelopment of the lymph system in general. Increased atresia of the ovarian follicles was observed. No other evidence of tissue changes could be noted.

Experiments with guinea pigs (Reyniers, *loc. cit.*) indicate that they are considerably more difficult to rear in germ-free systems. Animals on Purina rabbit chow developed normally for twenty-one days and showed a slow weight gain up to thirty-five days, after which growth ceased and death occurred at about sixty days. Appetites were reportedly ravenous and physical activity marked

until shortly before death. Postmortem examination indicated diminution of prenatal fat stores, atrophy of muscles, and weakness of bone structure. Skin and visible mucosae became anemic in appearance early in the experiment, and alopecia was prevalent. Cecums became greatly enlarged, reaching over 40 per cent of the total body weight, compared with the normal 15 per cent. The general picture resembled simple starvation except for the enlargement of the cecum and hunger symptoms.

In summarizing their long report on germ-free animals and in evaluating its potential significance to nutrition, the Indiana group has made several interesting points. They emphasize that germ-free animals exist on a unique biologic level and should be considered as new experimental material which must be studied at length before it can be properly used. There are limitations in its use, not only because of the apparatus employed, but also because the germ-free animal has not lived with microorganisms, hence has fewer immunities. It cannot, therefore, be subjected promiscuously to microbes in the manner that these might be introduced onto an agar plate. With these precautions in mind, these animals might well be used for studies such as (1) animal nutritional requirements and animal metabolism in general; (2) studies of immunity and resistance to infection, especially since selected strains of microorganisms may be introduced into these animals in any desired combination; (3) nutrition in relation to aging; and (4) the etiology of diseases for which the infectious agents are not well known. The preliminary work of establishing diets and experimental technics appears to be largely accomplished, and rapid advances in our knowledge in this field should be forthcoming.

RIBOFLAVIN, THIAMINE, AND VITAMIN A IN DAIRY PRODUCTS

The Committee on Milk and Dairy Products of the American Public Health Association has published a report on "Food Values of Dairy Products as Affected by Methods of Handling in Production, Distribution, and Use" (*Am. J. Pub. Health* **37**, *1113 (1947)*). The report was restricted to the vitamins riboflavin, thiamine, and vitamin A, and under each nutrient the dairy products, milk, butter, cheese, and ice cream, are considered from the standpoint of factors which affect these nutrients.

Riboflavin: Although there is some evidence that increased riboflavin in the feed of cows can cause a temporary increase in the riboflavin content of milk, and that increased volume of milk may decrease its concentration, the riboflavin content of milk is relatively constant. It is suggested that this constancy is due to synthesis of riboflavin in the rumen of the cow. Several typical studies were reported to indicate the great losses of riboflavin which may occur on exposure of milk to sunlight. Increased alkalinity, temperature, and time of exposure accentuate this loss so that as much as three quarters of the riboflavin may be lost in three and one-half hours of direct sunlight. Interestingly, a survey showed that 70 per cent of all the milk delivered on a typical retail route was removed from the doorstep within five minutes of delivery, and only 3 per cent remained more than two and one-half hours. Contrary to expectation, the ultraviolet irradiation of milk to increase its vitamin D content caused only negligible losses of riboflavin.

No losses of riboflavin have been noted during pasteurization, evaporation, or drying of milk. Storage of condensed milk except under considerably elevated temperatures causes little loss. Variations in cheese making are great, but a summary of reports indicate that one fourth to one third of the

riboflavin in the milk is retained in the cheese. As expected, the riboflavin is stable in ice cream and is equivalent to the cream that goes into the product.

Thiamine: As in the case of riboflavin, there appears to be little variation in the thiamine content of milk with variations in the rations of the cow, and it is recognized that this vitamin is also synthesized in the rumen. Losses of thiamine during pasteurization reportedly vary from 10 to 25 per cent in the holding process and appear negligible in high temperature-short exposure pasteurizations. One study indicated an 8 per cent loss in the boiling involved in preparing baby formulas and a one-third loss during the production of evaporated milk. Insignificant losses occurred during the drying of milk and its storage even at elevated temperature. Although no destruction of thiamine occurs during the manufacture of cheese, its high solubility results in a great loss in the whey expressed from the curd, hence only 8 to 15 per cent of the original thiamine was found to be retained in the finished cheese. Reports have been conflicting on the losses of thiamine during the ripening of cheese.

Vitamin A: Extensive studies carried on cooperatively in different experiment stations and federal laboratories leave no doubt that the carotene content of the ration of the cow greatly affects the vitamin A content of the butter produced. Thus of thousands of samples analyzed, "winter" butter contained an average of 11,000 I.U. per pound and "summer" butter 18,000 I.U. per pound. Calculated on similar fat contents, "summer" and "winter" milk vary in the same ratio. These marked differences support the value of studies designed to retain the carotene during processing and storage of forage crops for winter feeding.

Vitamin A appears to be very stable during the storage of butter, or during exposure of milk to sunlight or ultraviolet irradiation, and little loss of vitamin A has been reported in the pasteurization, evaporation, or drying of milk. In the manufacture of Cheddar cheese, one report indicates a retention of 85 per cent of the vitamin A, and little or no loss during storage.

The outstanding points summarized in regard to variations in these vitamins in dairy products are the changes which may occur in riboflavin in milk during exposure to sunlight and the relation of vitamin A content to the rations of the dairy cow. Other than these, no special precautions appear necessary to preserve the riboflavin, thiamine, and vitamin A in dairy products.

VITAMIN DEFICIENCY AND IMMUNITY IN RATS

The normal physiologic response of an animal to repeated injections of a foreign protein or other antigen is the production of antibodies, which appear in the blood serum and are capable of reacting with or neutralizing the stimulating antigen. The antibodies produced may constitute a significant proportion of the total serum proteins. The ability of an animal to form antibodies in response to an antigenic stimulus might thus be used as an additional criterion of adequate nutrition.

Previous results (*Nutrition Reviews* **5,** *111 (1947);* H. C. Stoerck, H. N. Eisen, and H. M. John, *J. Exp. Med.* **85,** *365 (1947)*), obtained by following the production of circulating antibodies in rats following injection of washed sheep erythrocytes, showed that the ability of pyridoxine deficient animals to elaborate antibodies was greatly impaired; normal serum antibody concentrations were, however, obtained in thiamine, riboflavin, and pantothenic acid deficient rats.

A. E. Axelrod, B. B. Carter, R. H. McCoy,

and R. Geisinger (*Proc. Soc. Exp. Biol. Med.* **66,** *137 (1947)*) have continued this investigation. Rats were fed a ration containing purified casein 25, sucrose 57, salts 4, hydrogenated vegetable oil 10, and corn oil 2, and supplemented with adequate amounts of the fat and water soluble vitamins. Individual groups deficient in pyridoxine, pantothenic acid, or thiamine, were obtained by omitting the appropriate single vitamin from the vitamin supplement. After seven weeks on the various rations, animals in the three vitamin deficient groups had ceased growing. At this point, immunization of deficient and control groups was begun. Human erythrocytes in a 10 per cent suspension in normal saline were used as antigen. An initial intraperitoneal injection of 0.5 ml. of the antigen suspension was followed on alternate days by five additional injections of 1 ml. each. Five days after the final injections the anesthetized animals were bled, the serum collected, and tested for their agglutinin titer against the antigen. A marked diminution in the antibody content of serums from the pyridoxine and pantothenic acid deficient animals was evident. In the former group, only one of 10 animals had a measurable agglutinin titer; in the latter group only one of 7 animals had such a titer. This failure to generate antibodies was not due to the low food intake of the deficient animals, since "inanition controls," whose food intake was limited to that of the deficient animals, exhibited high agglutinin titers only slightly less than animals fed the complete ration ad libitum. Riboflavin deficient animals showed antibody production intermediate in magnitude between that of the controls and that of the pantothenic acid and pyridoxine deficient rats.

The same conclusions with respect to antibody production were drawn from a determination of the hemolysin titers of serums from the various groups of animals. In these tests, guinea pig serum was used as a source of complement.

With our present knowledge of the intimate role played by pyridoxine in catalyzing transformations of amino acids (*Nutrition Reviews* **5,** *98 (1947)*), the apparent necessity of this vitamin for antibody (protein) production shown by this work and previous investigations (*loc. cit.*) is not surprising. The present investigations would indicate that pantothenic acid is important to almost the same degree, and riboflavin to a lesser extent. One may wonder why, in previous investigations (Stoerck and co-workers, *loc. cit.*), a deficiency in pantothenic acid or in riboflavin did not reduce the circulating antibodies. No entirely satisfactory explanation for this discrepancy is offered by the present authors. They do point out, however, that sheep erythrocytes, used as the antigen by Stoerck *et al.*, produce a much smaller response in terms of agglutinin titers in rats, than do the human erythrocytes used in the present investigation, and that where the antigenic stimulus and consequent antibody response is of this low order of magnitude, pantothenic acid and riboflavin may not become limiting factors in the production of circulating antibodies. Both groups of investigators emphasize that the decreased antibody titer in serum is not unequivocal proof for an impairment in antibody synthesis in tissues, since the possible effects of vitamin deficiency on distribution and destruction of antibodies are unknown and have not been determined. In view of these interesting results, however, it would appear that extreme deficiencies of pyridoxine and pantothenic acid might produce heightened susceptibility and lowered immunity to infections of various types. Clearcut deficiencies of these two vitamins in man have not been observed; the clinical significance of these observations is, therefore, a problem for further research.

INFLUENCE OF CHOLINE IN MEGALOBLASTIC ANEMIAS

Although experimental studies in animals have shown the effects choline deficiency may produce, the place of choline in human disorders is unknown. Consequently, interest was aroused by the report of F. B. Moosnick, E. M. Schleicher, and W. E. Peterson (*J. Clin. Invest.* **24,** *278 (1945)*) dealing with observations in a patient with pernicious anemia. This man had been successfully treated for several years with intramuscular injections of liver extract but eventually relapse occurred despite high dosage of refined liver extract. Liver biopsy showed acute hepatitis and moderately severe fatty changes. Choline chloride was administered intravenously for sixteen days in a daily dose of 1 g. This was followed by a reticulocyte response of 5.5 per cent and a progressive rise in the red cell count. In explanation of these events, it was suggested that the patient already had an adequate supply of hematopoietic substance stored in the liver but that this could not be elaborated in sufficient amounts on account of the fatty state of this organ, this dysfunction being corrected by administration of choline.

Stimulated by this report, L. J. Davis and A. Brown (*Blood* **2,** *407 (1947)*) investigated the role of choline chloride in the management of cases of pernicious anemia and other megaloblastic (macrocytic) anemias refractory to parenteral liver therapy. The quantity of choline chloride used varied from 3 to 10 g. when given orally and from 1 to 10 g. when given intravenously.

In one case of untreated pernicious anemia the administration of choline was without significant effect, while intravenous choline appeared to be followed by renewed red cell production in 2 cases in which the reticulocyte and red cell response to liver extract were initially suboptimal. The authors would have to admit that many more cases would need to be studied before the variable red blood cell responses which they noted could be accepted as being due to the action of choline.

In one case of megaloblastic anemia of pregnancy refractory to parenteral treatment with liver extract, the administration of choline was without effect. The subsequent administration of liver extract by mouth was followed by excellent blood regeneration, an observation which has been made in similar cases not treated with choline. In another case the administration of liver extract intramuscularly was followed by blood regeneration. The slight reticulocyte and erythrocyte count rise which followed the subsequent administration of choline were of such a small order as to cast doubt that they were more than fortuitous. In 2 cases of megaloblastic anemia associated with the sprue syndrome the intramuscular injection first of liver extract and secondly of choline was not followed by blood regeneration, but subsequent oral liver extract resulted in a clearcut hematopoietic response. This is a strange effect in a case of sprue, a disorder in which others have observed failure to respond to oral therapy and good response to parenteral liver therapy.

In a case of nutritional megaloblastic anemia, neither parenteral liver extract therapy nor choline was associated with any hematopoietic effect but the subsequent administration of folic acid was followed by striking blood regeneration. Finally, in 2 cases of refractory megaloblastic anemia of uncertain origin, the administration of choline chloride was associated with reticulocyte responses and red cell regeneration. These 2 cases, particularly the second, were similar to the case described by Moosnick *et al.* (*loc. cit.*) for earlier injections of liver extract had produced no response. Unfortunately liver biopsies were not performed and the sternal marrow smears provided no evidence of gross fatty changes as Moosnick and his associates found in their case.

From these observations the authors conclude that choline by itself is incapable of exerting a significant erythropoietic effect in megaloblastic anemias, but is capable of exerting a "boosting" effect in cases in which a response to parenteral liver therapy has already occurred. In cases of anemia refractory to parenteral liver therapy but responding to oral liver extract, the response does not seem to be influenced by choline. A second group of refractory cases may exist, however, of which the case of Moosnick *et al.* and the last 2 cases of Davis and Brown may be examples. In such cases the administration of choline, they believe, is effective. Davis and Brown suggest that if the essential defect in pernicious anemia is an inability on the part of the organism to liberate free folic acid from inactive conjugates assimilated from the food as A. D. Welch, R. W. Heinle, E. M. Nelson, and H. V. Nelson (*J. Biol. Chem.* **164,** *787 (1946)*) have suggested, and if the hematopoietic activity of liver extract is concerned with the restoration of this function, it may be that the site of this action is in the liver and that in some way the administration of choline influences this activity of the liver. Unfortunately, Davis and Brown offer no evidence of hepatic dysfunction in their cases. Their thesis is particularly difficult to accept in view of the well known functional reserve of the liver and the well established observation that adequate function of the liver is compatible even with extensive pathologic changes.

In considering the observations recorded by these investigators one is left with the conclusion that there are many aspects of megaloblastic anemia which are not understood. Their observations make it clear that the role of choline chloride in the management of refractory cases of megaloblastic anemia deserves further study.

COMPARISON OF FOLIC ACID AND LIVER THERAPY IN MACROCYTIC ANEMIAS

Somewhat more than two years have elapsed since the first report of the effect of pteroylglutamic (folic) acid in the treatment of macrocytic anemia (pernicious anemia, nutritional macrocytic anemia, and sprue). Evidence is accumulating which indicates that at least in certain cases of pernicious anemia, folic acid therapy does not replace liver therapy. It has also been reported that in certain cases of macrocytic anemia administration of folic acid may result in blood regeneration where liver therapy has failed.

R. W. Heinle, J. T. Dingle, and A. S. Weisberger (*J. Lab. Clin. Med.* **32,** *970 (1947)*) report their observations in 41 patients with proved macrocytic anemia, in 35 of whom it was possible to compare the results of folic acid therapy with previous antianemia therapy with intramuscular injections of crude and purified liver extracts, oral liver-stomach therapy, and stomach therapy. The folic acid was administered parenterally to 25 patients and orally to 16 patients. The parenteral dose was 50 mg. in 15 patients and 150 mg. in 9 patients.

Of the whole series, 32 patients were studied six to twelve months (average of ten and seven-tenths), the remainder having been observed less than six months. Of the 32, 72 per cent maintained satisfactory erythrocyte counts and hemoglobin concentrations. Six patients of the entire series of 41 showed definite improvement in erythrocyte values as compared with previous liver extract therapy. With the exception of one patient, those showing improvement were either on daily oral folic acid therapy or were receiving intramuscular injections at intervals of two weeks. Analysis of the data suggests that the improvement obtained with folic acid therapy occurred in patients who had not been receiving enough liver extract previously or were not absorbing

liver extract efficiently enough from the gastrointestinal tract to maintain normal blood levels.

In 2 of the patients a moderate decrease in erythrocyte count (300,000 to 500,000 per cubic millimeter) occurred, while in 7 the decrease was greater than 500,000 per cubic millimeter. It is noteworthy that in all these cases injections of folic acid were at intervals of three to six weeks. The size of the individual dose was not correlated with the severity of the relapse. None of the patients receiving oral therapy with folic acid showed a hematologic relapse. The history of one of these cases suggests that repeated short periods of improvement followed folic acid therapy which was succeeded by increasing degrees of relapse in the intervening intervals of four weeks. This observation suggests that folic acid is not stored well and is consistent with the observations of R. Steinkamp, C. F. Shukers, J. R. Totter, and P. L. Day (*Proc. Soc. Exp. Biol. Med.* **63,** *556 (1946)*) who showed that a large part of administered folic acid is excreted in a relatively short time.

Outright failure to respond satisfactorily to folic acid was observed in one patient. Successive periods of ten days' duration in which the patient was given daily intramuscular injections of 1 mg. of folic acid, 6 mg., 12 mg., and 12 mg. together with 36 mg. of xanthopterin produced slight reticulocyte responses but no regeneration of red cells. The subsequent daily intramuscular injection of 15 units of purified liver extract produced a theoretically maximal reticulocyte response and a rapid increase in erythrocytes and hemoglobin.

Of serious import is the fact that neurologic relapse occurred in 3 of the 41 patients. This was observed in one patient whose reticulocyte response to folic acid therapy had been substantially greater than the theoretical maximum and the erythrocyte and hemoglobin regeneration had also been good. Eighty-three days after beginning treatment there was a sudden and rapidly progressive onset of paresthesias with rapid loss of deep reflexes and vibratory sensation. Liver extract therapy for twenty-seven days was necessary to produce the first evidence of arrest of the neurologic lesions. Relapse of similar though less pronounced character was observed in 2 other patients.

The three instances in which folic acid therapy proved useful after liver treatment had failed, which J. Waldenström describes (*Blood* **2,** *426 (1947)*), include only one case of true pernicious anemia. The first case unlike true pernicious anemia had gastric anacidity and, in addition, had glossitis, difficulty in swallowing, and perlèche, and both the liver and spleen were enlarged. She had been successfully treated with liver extracts for twelve years, but suddenly became refractory with a marked drop in the erythrocyte and white cell counts. Folic acid in large doses had an excellent effect and the patient's blood has been normal for about a year without further treatment of importance. Waldenström regarded his second case as one of idiopathic steatorrhea although the case was by no means typical, the patient having a large spleen and pronounced leukopenia. Free hydrochloric acid was present in the gastric contents. After unsuccessful liver therapy, improvement followed the administration of folic acid. Only the third case really resembled pernicious anemia although even massive doses of liver extract failed to bring the erythrocyte or hemoglobin values to normal. The administration of folic acid was associated with substantial improvement.

It would seem likely from these observations that the modes of action of folic acid and of liver extract are not identical. Thus cases may be encountered occasionally in which only one or the other agent will be effective. It is also becoming increasingly evident from the observations cited above, from the report of C. F. Vilter, R. W. Vilter, and T. D. Spies (*J. Lab. Clin. Med.* **32,** *262 (1947)*), and those of others (Editorial, *New*

Engl. J. Med. **237,** *713 (1947)*) that folic acid therapy does not prevent the development or progress of changes in the nervous system in cases of pernicious anemia.

THE SITE OF CONVERSION OF CAROTENE TO VITAMIN A

T. Moore (*Biochem. J.* **25,** *275 (1931)*) reported some years ago that the livers from rats fed on large amounts of carotene (red palm oil or carrot fat) for periods of one to three months were very high in vitamin A content. These same livers contained little carotene. Conversely, the alimentary tract of these animals contained relatively large amounts of carotene and only small quantities of vitamin A. These observations were interpreted by Moore to indicate that the conversion of carotene to vitamin A probably takes place in the liver. This interpretation has been widely accepted and is consistent with much data in the literature.

Some recent observations, however, indicate that in the rat the conversion of carotene to vitamin A takes place in the intestinal wall rather than in the liver. F. H. Mattson, J. W. Mehl, and H. J. Deuel (*Arch. Biochem.* **15,** *65 (1947)*) studied the distribution of carotene and of vitamin A in the rat following oral administration of a solution of partially purified beta-carotene. Twenty-one day old rats of standardized nutritional background were placed on the USP XII vitamin A deficient diet. After three weeks, chemical analysis of the various tissues showed that the animals retained no appreciable stores of vitamin A.

Vitamin A depleted control rats were fed by stomach tube 0.5 ml. of cottonseed oil containing 0.5 per cent tocopherol. The animals were killed at hourly intervals and analyses of the livers and intestinal tracts were carried out. Despite the absence of carotene and vitamin A from the diet of these animals, a small amount of "apparent carotene" and "apparent vitamin A" was found in both liver and intestine. This amounted to an average total of 1 to 2 micrograms of "apparent carotene" in the liver or gastrointestinal tract and 1 to 2 I.U. of "apparent vitamin A" in the intestinal tract or liver. The properties of these chromogens were different from those of true carotene or vitamin A.

Similar vitamin A depleted animals were supplemented with a single dose of a fish liver oil containing approximately 12,000 I.U. of vitamin A and then killed at intervals of two to four days. The subsequent analyses indicated that the concentration of vitamin A in the liver increased to a level of 800 or 900 I.U. per gram by the third day and remained at this level for a period of two weeks or more. Simultaneously, the vitamin A content of the gastrointestinal tract was highest the first day after administration of a fish liver oil and decreased until by the end of the first week the vitamin had disappeared from the intestine. These observations indicated that the vitamin A content of the gastrointestinal tract was not elevated above that of control deficient rats despite the higher liver stores of the treated animals.

Approximately 900 micrograms of carotene were given by stomach tube to a third series of animals previously depleted of vitamin A. The rats were then killed at fifteen to thirty minute intervals and again the vitamin A and carotene distributions of the gastrointestinal tract and liver determined chemically. Only apparent vitamin A was found in the livers of those animals killed during the first two and a half hours after the feeding of carotene. The concentration of this chromogen was similar to that observed in the control animals. This apparent vitamin A could be distinguished from the true vitamin A because the apparent vitamin A developed an amber color instead of the usual blue color with the Carr-Price reagent. In the gastrointestinal tract, however, small but definite amounts of vitamin A appeared during these first two and a half hours. The quantities found

ranged from a concentration of 2.38 to 3.89 I.U. per gram or a total of 6.03 to 8.57 I.U. per animal. After two and a half hours, the content of vitamin A increased in the liver as well as in the intestinal tract. It was not, however, until after four hours that the concentration of vitamin A in the liver became greater than the concentration of vitamin A in the intestinal tract. No appreciable consistent increase in total liver carotene was found. To test whether the small amount of apparent carotene in the liver might be true carotene, these investigators administered approximately 1800 micrograms of carotene to each of 25 vitamin A deficient animals. The rats were killed seven hours later and the pooled livers extracted. The absorption curve of the pigments so obtained had a maximum in the ultraviolet range and no inflection at 450 mμ. Other properties indicated that this pigment was not a carotenoid.

These several observations lead the workers to conclude that the site of conversion of carotene to vitamin A in the rat is the intestinal wall.

To test this conclusion further, C. E. Wiese, J. W. Mehl, and H. J. Deuel (*Arch. Biochem.* **15,** *75* (*1947*)) studied *in vitro* the transformation of carotene to vitamin A by the small intestine of the vitamin A deficient rat. Vitamin A depleted animals were prepared as indicated above. Three hundred and ten micrograms of carotene were administered by stomach tube and then washed immediately from the stomach into the intestine with Ringer-Locke solution. The intestine was then ligated at the pylorus and ileocecal valve, removed, and incubated anaerobically in Ringer-Locke solution for three hours at 37°C. The intestinal contents were then removed, washed, and the intestinal wall saponified, extracted, and the vitamin A determined by the Carr-Price reaction. In eighteen individual experiments an average of 8.7 I.U. of vitamin A was found in the gut wall after the incubation of the intestines of vitamin A deficient rats with the carotene solution. No vitamin A could be demonstrated (1) in unincubated intestines of vitamin A deficient rats, (2) in intestines of vitamin A deficient rats to which carotene had been administered but which had then been immediately flushed with saline solution, (3) in intestines of vitamin A deficient rats incubated with the vehicle in which the carotene was administered, or (4) in a bath in which carotene was incubated with Ringer-Locke solution in absence of intestine. The authors' conclusion seems justified that the transformation of carotene to vitamin A has been demonstrated to take place *in vitro* in the small intestine of the vitamin A deficient rat. They did not report upon attempts to determine whether the liver of the vitamin A deficient rat has the ability to convert carotene into vitamin A *in vitro*.

These reports indicate that the intestinal wall can convert carotene into vitamin A, a transformation which appears to occur *in vivo* in the intestinal wall under the conditions of the experiments. It is to be noted, however, that these studies were acute ones and do not indicate whether the intestine is the sole site of conversion of carotene into vitamin A in the rat. In a previous paper, however, E. L. Sexton, J. W. Mehl, and H. J. Deuel (*J. Nutrition* **31,** *299* (*1946*)) demonstrated that parenterally administered carotene was deposited in the liver of rats and that it was still present in considerable quantities as long as forty-six days after injection. Following the administration of carotene, no increase in vitamin A content of the liver was observed. It would seem unlikely, therefore, that the liver could play an important role in the conversion of carotene to vitamin A in the rat unless the results with parenterally administered carotene were peculiarly influenced by the state of dispersion of the injected carotene.

A report by R. M. Johnson and C. A. Baumann (*Arch. Biochem.* **14,** *361* (*1947*)) may have further bearing on the question of the organ concerned with the formation of

vitamin A. These workers administered a number of isomers of carotene to vitamin A depleted rats and determined the distribution of carotene and of vitamin A in several organs. Their studies differed from those of the California investigators in that the carotenes were administered over a fifteen day supplementation period in daily doses of from 20 to 80 micrograms. They found that at low levels of carotene intake, i.e. 35 I.U. daily, more vitamin A appeared in the kidney than in the liver. At higher levels of intake, however, more vitamin A appeared in the liver than the kidney. There was no evidence of any accumulation of carotenoids in either the liver or the kidney when these various isomers were fed, and it was noted that the total amounts of ether soluble yellow pigment were essentially the same whether a large quantity of carotene or none at all was given. These observations would indicate that although the kidney takes up vitamin A rapidly it is probably not a site of conversion of the precursor to the vitamin.

EXCRETION OF B-COMPLEX VITAMINS IN INFANCY

The intermediary metabolism characteristic of the newborn infant still remains in large part a mystery despite extensive investigations of the subject for many years. B. M. Hamil *et al.* (*Am. J. Dis. Child.* **74,** *434 (1947)*) contributed data concerning the urinary excretion of certain vitamins in infants and interpreted their observations as they may apply to the specialized metabolism of the infant.

The infants studied during the first seven days of life were born during March, April, and May from healthy mothers following uncomplicated pregnancy and normal delivery. They were breast-fed during the period of study. Urine specimens were collected as passed; collection of twenty-four hour specimens being abandoned. Ascorbic acid, thiamine, riboflavin, niacin, pantothenic acid, and biotin were measured microbiologically. The two methods for riboflavin were in fair agreement and showed similar changes from specimen to specimen. In the case of ascorbic acid the authors not only studied the excretion during the first seven days of life, but also determined the ascorbic acid content of cord and maternal blood, and of the infants' blood daily.

The ascorbic acid content of the bloods varied widely. Thus the cord blood values ranged from 0.66 to 2.18 mg. per 100 ml., the maternal serum from 0.04 to 1.19 mg. per 100 ml., and the infants' blood during the first week of life from 0.20 to 1.72 mg. per 100 ml.

The urinary excretion of all the vitamins (including N′-methylnicotinamide) was high during the first few days of life and then at varying times rather sharply fell off to low levels. For example, the maximal excretion of thiamine and pantothenic acid was on the third day, of riboflavin on the first day, of niacin, N′-methylnicotinamide, and biotin on the second day. All fell off markedly by the seventh day of life.

In interpreting these data the authors consider unlikely the possibility that the later low values are the result of increase in the metabolic processes coincident with growth and rapid weight gain, but suggest that the high excretion on the first few days may be the result of the change from an aquatic to gaseous environment or that vitamins needed in excess in the relatively anoxic intrauterine state are lost shortly after birth, or finally, that the change in the renal function at birth may be a factor in the excess excretion of the vitamins. The possibility that the excess excretion of the vitamins is the result, at least in part, of a high fluid intake during the period of loss of weight immediately following birth is considered by the authors and, as they point out, might indicate a "washing out" of the water soluble vitamins.

NOTES

Choline and Anemia

In view of the clinical use of choline in attempts to modify fatty or cirrhotic changes in the liver, the reports of J. E. Davis and co-workers (see *Nutrition Reviews* **4,** *83* (*1946*)) that fat plus choline or choline itself may cause a macrocytic anemia, are of special interest. In the review referred to it was noted that the amount of choline administered either to dogs or human beings was only a fraction of that present in the basal diet, and furthermore that the blood regeneration picture was not entirely clear. It appeared therefore that choline administered in the free form might have special effects which are absent with dietary choline, and in fact Davis later reported (*Science* **105,** *43* (*1947*); *Nutrition Reviews* **5,** *148* (*1947*)) that the effect of choline was through a vasodilator effect on the bone marrow since atropine inhibited the choline anemia.

On the other hand, M. F. Clarkson and C. H. Best (*Science* **105,** *622* (*1947*)) have been unable to confirm the original observations of Davis and co-workers. In their experiments, 2 dogs were given 10 mg. of choline per kilogram of body weight plus 60 g. of lard per day for eight days in addition to the basal diet (all at one feeding), and 2 other dogs were given 30 mg. of choline per kilogram of body weight per day, but in three divided doses. The latter treatment was continued for forty-five days. The rather immediate response in the blood picture noted by Davis and co-workers was entirely absent.

According to the calculations of Clarkson and Best, the basal diet employed by them contained somewhat more choline, but otherwise it is difficult to trace the difference in the results of the two groups of investigators. Pending further reports it can only be assumed that the anemia-producing effect of choline is at least restricted to special conditions and is not the inevitable result of choline administration.

Alloxan Diabetes and Vitamins

The etiology of diabetes is still obscure, but the experimental production of diabetes in animals by alloxan has aroused considerable speculation on the metabolic origin of this disease. J. S. Dunn, J. Kirkpatrick, N. G. B. McLetchie, and S. V. Telfer (*J. Path. Bact.* **55,** *245* (*1943*)) suggest that possible defects in the metabolism of purines may be concerned.

A possible relation of the vitamins to the production of alloxan diabetes is indicated by the work of S. Banerjee (*Science* **106,** *128* (*1947*)) who observed that niacin and two of its derivatives, pyridine-dicarboxylic acid and 2-phenylquinoline-4-carboxylic acid, prevent the diabetogenic action of alloxan. The investigations were initiated by the observation that 1,2-dimethyl-4-amino-5 (d-1^1-ribityl amino)-benzene, which condenses with alloxan *in vitro* to form riboflavin, also prevented the diabetogenic action of alloxan *in vivo*. A similar condensation *in vivo*, for which there was some evidence, might explain the action of this riboflavin derivative. However, the preventive effect of niacin and its two derivatives, each possessing a carboxyl group and the pyridine ring, may have special significance. Calcium pantothenate, glycine, benzoic acid, para-aminobenzoic acid, ascorbic acid, and pyridoxine showed no preventive action toward the diabetogenic effect of alloxan.

In evaluating the effect of niacin or its similarly acting derivatives, it must be pointed out that these compounds are effective only when administered with the alloxan; they do not reverse the effect of alloxan once established. The preventive dose of niacin was large, roughly equimolecular with the alloxan, hence it may have little to do with niacin as a vitamin. Vitamins are, after all, chemical compounds which may have pharmacodynamic and chemical properties independent of their

nutritional activity. Nevertheless, the conclusion of Banerjee that niacin, when sufficient in the diet, might detoxify naturally formed alloxan or similar products of purine metabolism, is at least worth consideration in a study of the etiology of diabetes.

Adequacy of Diet and Speed of Rachitogenesis

Most official methods for determining vitamin D with rats utilize some modification of the Steenbock ration as the rachitogenic diet. This ration is composed largely of yellow corn and wheat gluten, with added calcium carbonate and salt. On such a ration, growth is slow, and a depletion period of eighteen to twenty-five days is required to produce a deficiency suitable for subsequent use in assay of vitamin D.

Since the rate of growth during the depletion period of rats on such a ration is slow (as little as 0.25 g. per day with some animals) (P. S. Francis, *J. Assn. Official Agr. Chem.* **30**, *364* (*1947*)), and since rickets can only develop while there is bone growth, some of the individual animals do not develop satisfactory rickets during the depletion period. In attempting to improve the growth rate on the diet, Francis, from published figures noted that the lysine furnished by the Steenbock ration (0.5 per cent) was only about half that found by W. C. Rose (*Science* **86**, *298* (*1937*)) to be necessary for the rat (1.0 per cent). All other essential amino acids were present in ample amounts. The deficit in lysine was verified by direct microbiologic assay of the ration. Subsequent experiments showed that addition of 0.5 per cent of lysine to the ration nearly doubled the growth rate of rats during the depletion period. With increased growth, rickets developed more rapidly. Comparative line tests following vitamin D therapy showed that rats depleted for sixteen days on the lysine-supplemented diet were as satisfactory for assay purposes as animals depleted for twenty-three to twenty-five days on the unsupplemented ration.

At present, pure lysine is too expensive to be incorporated into diets for routine vitamin D assays. Addition of sufficient blood fibrin to supply the additional amount of lysine led to the same improved growth observed with lysine, but atypical and irregular calcification of a type unsuitable for assay work occurred on this ration. Further work will be required to find a suitable natural source of lysine.

The work emphasizes how an exact knowledge of the composition of natural foodstuffs will be of aid to nutritionists in predicting the adequacy of natural rations. It is also interesting that use of a more complete ration in this instance has increased the speed with which a dietary deficiency becomes evident by increasing the rate of growth, and consequently the requirement for a substance still lacking from the diet.

Oxford Nutrition Survey

The Oxford Survey group, under the direction of Dr. H. M. Sinclair, has been given departmental status in Oxford University. The new organizational plans will afford greater stability and will facilitate close cooperation with the Ministry of Health.

Compounds Formed from Molds

A comprehensive collection of compounds which may be elaborated from sugars by molds has been published (B. S. Gould, *Scientific Report Series* **7**, *Sugar Research Foundation, Inc., New York* (*June*) (*1947*)). The summary includes one hundred and fifteen compounds, divided into mold pigments, polysaccharides, and antibiotic agents. Each listing is accompanied by a reference to the organisms which produce the compound, the yields obtained, and important literature references. This collection should be of value to teachers and research workers in this rapidly expanding field.

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